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UTILITY PATENT APPLICATION TRANSMITTAL
(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	1101-226	Total Pages	151
First Named Inventor or Application Identifier			
Vernon L. Alvarez			
Express Mail Label No.	EM 061 024 120 US		

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

1. ☒ Fee Transmittal Form (2 pages, in duplicate) [Total Pages 4]
   Submit an original, and a duplicate for fee processing

2. ☒ Specification [Total Pages 94]
   (preferred arrangement set forth below)
   -Descriptive title of the Invention
   -Cross Reference to Related Applications
   -Statement Regarding Fed sponsored R&D
   -Reference to Microfiche Appendix
   -Background of the Invention
   -Brief Summary of the Invention
   -Brief Description of the Drawings *(if filed)*
   -Detailed Description of the Invention (including drawings, *if filed*)
   -Claim(s)
   -Abstract of the Disclosure

3. ☒ Drawing(s) *(35 USC 113)* [Total Sheets 9]

4. ☒ Oath or Declaration [Total Sheets 2]
   a. ☐ Newly executed (original or copy)
   b. ☒ Copy from a prior application (37 CFR 1.63(d))
      (for continuation/divisional with Box 17 completed)
      [Note Box 5 below]
      i. ☐ DELETION OF INVENTORS(S)
         Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33 (b).

5. ☒ Incorporation By Reference *(useable if Box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

ADDRESS TO: Assistant Commissioner for Patents
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Washington, DC 20231

6. ☐ Microfiche Computer Program *(Appendix)*

7. ☒ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Copy
   b. ☒ Paper Copy (identical to computer copy) [Total Pages 40]
   c. ☐ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))

9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure ☐ Copies of IDS
    Statement (IDS)/PTO-1449 Citations

12. ☐ Preliminary Amendment

13. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*

14. ☒ Small Entity ☒ Statement filed in prior application,
    Statement(s) Status still proper and desired [Total Pages 2]

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Other:

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17. **If a CONTINUING APPLICATION**, *check appropriate box and supply the requisite information:*
   ☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: 09/273,685 filed March 22, 1999.

18. CORRESPONDENCE ADDRESS

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: ☒ Application of: Alvarez
    ☐ Patent of:

☒ Serial No.: 08/488,161   Group Art Unit:
☐ Patent No.:

☒ Filed: June 7, 1995   Examiner:
☐ Issued:

For: ANTIGEN BINDING PEPTIDES (ABTIDES)   Attorney Docket No.: 1101-176
      FROM PEPTIDE LIBRARIES

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
[37 CFR 1.9(f) and 1.27(c)] - Small Business Concern

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby declare that I am

        ☐ the owner of the small business concern identified below:
        ☒ an official of the small business concern empowered to act in behalf of
        the concern identified below:
        Name of concern   Cytogen Corporation
        Address of concern 600 College Road East
                      Princeton, NJ 08540

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the person employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern and/or there is an obligation under contract or law by the inventor(s) to convey rights to the small business concern with regard to the invention, entitled "ANTIGEN BINDING PEPTIDES (ABTIDES) FROM PEPTIDE LIBRARIES" by inventor(s) Alvarez described in

        ☐ the specification filed herewith
        ☒ application serial no. 08/488,161 filed June 7, 1995.
        ☐ patent no. issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no

rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME_____

ADDRESS_____

☐ INDIVIDUAL   ☐ SMALL BUSINESS CONCERN   ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28 (b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, or any patent to which this verified statement is directed.

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Name of person signing_____ Richard J. Walsh_____

Title of person other than owner__ Vice President of Corporate Development_____

Address of person signing_____ 600 College Road East_____

_____ Princeton, NJ 08540_____

Signature_____ Date_ 8/4/5 _____

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

ANTIGEN BINDING PEPTIDES (ABTIDES)
FROM PEPTIDE LIBRARIES

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ANTIGEN BINDING PEPTIDES (ABTIDES)
FROM PEPTIDE LIBRARIES

This application is a continuation-in-part of co-pending U.S. Patent Application Serial No. 08/310,192 filed September 21, 1994, the entire contents of which are incorporated herein by reference.

1. FIELD OF THE INVENTION

The present invention relates generally to peptides capable of specific binding to ligands of interest. The present invention also relates to peptides capable of mimicking the specific binding of a receptor to its ligand, an antibody to its antigen, and the like. Such peptides are known as "abtides." Abtides are identified by first and second screening steps of peptide libraries. The first screening step uses an antibody or receptor as a first target ligand and identifies peptide sequences ("mimetopes") which specifically bind to the antibody or receptor. The mimetopes are then incorporated into a second target ligand in a second screening step to identify abtides that bind the mimetope. Abtides mimic the binding specificity of the antibody (to its antigen) or the receptor (to its ligand) that was used as the first target ligand in the first screening step. The invention further relates to the use of abtides in the place of antibodies in assays. The invention also provides abtide compositions for use in therapy and diagnosis of disease.

2. BACKGROUND OF THE INVENTION

2.1. PEPTIDE LIBRARIES

The use of peptide libraries is well known in the art. Such peptide libraries have generally been constructed by one of two approaches. According to one approach, peptides have been chemically synthesized *in vitro* in several formats. For example, Fodor et al., 1991, Science 251: 767-773, describes use of complex instrumentation, photochemistry and

computerized inventory control to synthesize a known array of
short peptides on an individual microscopic slide. Houghten
et al., 1991, Nature 354: 84-86, describes mixtures of free
hexapeptides in which the first and second residues in each
5 peptide were individually and specifically defined. Lam et
al., 1991, Nature 354: 82-84, describes a "one bead, one
peptide" approach in which a solid phase split synthesis
scheme produced a library of peptides in which each bead in
the collection had immobilized thereon a single, random
10 sequence of amino acid residues. For the most part, the
chemical synthetic systems have been directed to generation of
arrays of short length peptides, generally fewer than about 10
amino acids or so, more particularly about 6-8 amino acids.
Direct amino acid sequencing, alone or in combination with
15 complex record keeping of the peptide synthesis schemes, is
required to use these libraries.

   According to a second approach using recombinant DNA
techniques, peptides have been expressed in biological systems
as either soluble fusion proteins or viral capsid fusion
20 proteins.

   A number of peptide libraries according to the
second approach have used the M13 phage. M13 is a filamentous
bacteriophage that has been a workhorse in molecular biology
laboratories for the past 20 years. M13 viral particles
25 consist of six different capsid proteins and one copy of the
viral genome, as a single-stranded circular DNA molecule.
Once the M13 DNA has been introduced into a host cell such as
E. coli, it is converted into double-stranded, circular DNA.
The viral DNA carries a second origin of replication that is
30 used to generate the single-stranded DNA found in the viral
particles. During viral morphogenesis, there is an ordered
assembly of the single-stranded DNA and the viral proteins,
and the viral particles are extruded from cells in a process
much like secretion. The M13 virus is neither lysogenic nor
35 lytic like other bacteriophage (e.g., λ); cells, once
infected, chronically release virus. This feature leads to
high titers of virus in infected cultures, i.e., 10^{12} pfu/ml.

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The genome of the M13 phage is ~8000 nucleotides in length and has been completely sequenced. The viral capsid protein, protein III (pIII) is responsible for infection of bacteria. In *E. coli*, ·the pillin protein encoded by the
5 F factor interacts with pIII protein and is responsible for phage uptake. Hence, all *E. coli* hosts for M13 virus are considered male because they carry the F factor. Several investigators have determined from mutational analysis that the 406 amino acid long pIII capsid protein has two domains.
10 The C-terminus anchors the protein to the viral coat, while portions of the N-terminus of pIII are essential for interaction with the *E. coli* pillin protein (Crissman and Smith, 1984, Virology 132: 445-455). Although the N-terminus of the pIII protein has been shown to be necessary for viral
15 infection, the extreme N-terminus of the mature protein does tolerate alterations. In 1985, George Smith published experiments reporting the use of the pIII protein of bacteriophage M13 as an experimental system for expressing a heterologous protein on the viral coat surface (Smith, 1985,
20 Science 228: 1315-1317). It was later recognized, independently by two groups, that the M13 phage pIII gene display system could be a useful one for mapping antibody epitopes. De la Cruz et al., 1988, J. Biol. Chem. 263: 4318-4322 cloned and expressed segments of the cDNA encoding the
25 *Plasmodium falciparum* surface coat protein into the pIII gene, and recombinant phage were tested for immunoreactivity with a polyclonal antibody. Parmley and Smith, 1988, Gene 73: 305-318 cloned and expressed segments of the *E. coli* β-galactosidase gene in the pIII gene and identified
30 recombinants carrying the epitope of an anti-β-galactosidase monoclonal antibody. The latter authors also· described a process termed "biopanning", in which mixtures of recombinant phage were incubated with biotinylated monoclonal antibodies, and phage-antibody complexes could be specifically recovered
35 with streptavidin-coated plastic plates.

In 1989, Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218 suggested that short, synthetic DNA segments

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cloned into the pIII gene might represent a library of epitopes. These authors reasoned that since linear epitopes were often ~6 amino acids in length, it should be possible to use a random recombinant DNA library to express all possible

5 hexapeptides to isolate epitopes that bind to antibodies.

Scott and Smith, 1990, Science 249:386-390 describe construction and expression of an "epitope library" of hexapeptides on the surface of M13. The library was made by inserting a 33 base pair Bgl I digested oligonucleotide

10 sequence into an Sfi I digested phage fd-tet, *i.e.*, fUSE5 RF. The 33 base pair fragment contains a random or "degenerate" coding sequence $(NNK)_6$ where N represents G, A, T or C and K represents G or T. The authors stated that the library consisted of 2×10^8 recombinants expressing 4×10^7 different

15 hexapeptides; theoretically, this library expressed 69% of the 6.4×10^7 possible peptides (20^6). Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87: 6378-6382 also described a somewhat similar library of hexapeptides expressed as pIII gene fusions of M13 fd phage. PCT publication WO 91/19818 dated December

20 26, 1991 by Dower and Cwirla describes a similar library of pentameric to octameric random amino acid sequences.

Devlin et al., 1990, Science, 249:404-406, describes a peptide library of about 15 residues generated using an (NNS) coding scheme for oligonucleotide synthesis in which S

25 is G or C.

Christian and colleagues have described a phage display library, expressing decapeptides (Christian et al., 1992, J. Mol. Biol. 227:711-718). The starting DNA was generated by means of an oligonucleotide comprising the

30 degenerate codons $[NN(G/T)]_{10}$ with a self-complementary 3' terminus. This sequence, in forming a hairpin, creates a self-priming replication site which could be used by T4 DNA polymerase to generate the complementary strand. The double-stranded DNA was cleaved at the Sfi I sites at the 5' terminus

35 and hairpin for cloning into the fUSE5 vector described by Scott and Smith, *supra*.

Other investigators have used other viral capsid proteins for expression of non-viral DNA on the surface of phage particles. The protein pVIII is a major M13 viral capsid protein and interacts with the single stranded DNA of

5 M13 viral particles at its C-terminus. It is 50 amino acids long and exists in approximately 2,700 copies per particle. The N-terminus of the protein is exposed and will tolerate insertions, although large inserts have been reported to disrupt the assembly of pVIII fusion proteins into viral

10 particles (Cesareni, 1992, FEBS Lett. 307:66-70). To minimize the negative effect of pVIII fusion proteins, a phagemid system has been utilized. Bacterial cells carrying the phagemid are infected with helper phage and secrete viral particles that have a mixture of both wild-type and pVIII

15 fusion capsid molecules. pVIII has also served as a site for expressing peptides on the surface of M13 viral particles. Four and six amino acid sequences corresponding to different segments of the *Plasmodium falciparum* major surface antigen have been cloned and expressed in the comparable gene of the

20 filamentous bacteriophage fd (Greenwood et al., 1991, J. Mol. Biol. 220:821-827).

Lenstra, 1992, J. Immunol. Meth. 152:149-157 described construction of a library by a laborious process encompassing annealing oligonucleotides of about 17 or 23

25 degenerate bases with an 8 nucleotide long palindromic sequence at their 3' ends. This resulted in the expression of random hexa- or octa-peptides as fusion proteins with the β-galactosidase protein in a bacterial expression vector. The DNA was then converted into a double-stranded form with Klenow

30 DNA polymerase, blunt-end ligated into a vector, and then released as Hind III fragments. These fragments were then cloned into an expression vector at the C-terminus of a truncated β-galactosidase to generate 10^7 recombinants. Colonies were then lysed, blotted on nitrocellulose filters

35 (10^4/filter) and screened for immunoreactivity with several different monoclonal antibodies. A number of clones were isolated by repeated rounds of screening and were sequenced.

Cull et al., 1992, Proc. Natl. Acad. Sci. USA
89:1865-1869 described a system in which random peptides were
fused to the carboxy terminus of the *lac* repressor. The
fusion proteins contained an intact *lac* amino terminus (which
5 is responsible for specific binding of the *lac* repressor to
the DNA sequences constituting the *lac* operator sites). The
nucleotide sequences encoding the fusion protein were cloned
into a plasmid containing copies of the *lac* operator site.
Thus, when the fusion protein was expressed in bacteria, it
10 became bound to the operator sites of the plasmid encoding it.
This provided a physical linkage between the fusion protein
and the gene encoding it. When bacteria containing the
plasmid were screened with ligands for which it was desired to
isolate binding partners, the fusion proteins comprising
15 peptides that specifically bound to the ligand were isolated,
carrying along the genes that encoded those fusion proteins.
        A comprehensive review of various types of peptide
libraries can be found in Gallop et al., 1994, J. Med. Chem.
37:1233-1251.
20

        2.2.   LIGANDS USED TO SCREEN PEPTIDE LIBRARIES
        Screening of peptide libraries has generally been
confined to the use of a restricted number of ligands. Most
commonly, the ligand has been an antibody (Parmley and Smith,
25 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990,
Science 249:386-390). In many cases, the aim of the screening
is to identify peptides from the library that mimic the
epitopes to which the antibodies are directed. Thus, given an
available antibody, peptide libraries are excellent sources
30 for identifying epitopes or epitope-like molecules of that
antibody (Yayon et al., 1993, Proc. Natl. Acad. Sci. USA
90:10643-10647).
        While previous studies have succeeded in identifying
epitopes and epitope-like molecules from peptide libraries, it
35 has not been realized in the prior art that this approach
could be extended by using the identified epitopes in a

further round of screening of a peptide library to identify antibody-like molecules.

When it has been desired to obtain antibody-like molecules, the prior art has employed peptide libraries that
5 contain naturally occurring antibody sequences. This has probably been due to the fact that specific binding by antibodies is known to depend upon a complex structure involving various complementarity determining regions (CDRs), often from both heavy and light antibody chains. Short
10 peptides would not be expected to mimic such structures and longer peptides were thought to be unsuitable for display in the most commonly used libraries.

McCafferty et al., 1990, Nature 348:552-554 used PCR to amplify immunoglobulin variable (V) region genes and cloned
15 those genes into phage expression vectors. The authors suggested that phage libraries of V, diversity (D), and joining (J) regions could be screened with antigen. The phage that bound to antigen could then be mutated in the antigen-binding loops of the antibody genes and rescreened. The
20 process could be repeated several times, ultimately giving rise to phage which bind the antigen strongly.

Marks et al., 1991, J. Mol. Biol. 222:581-597 also used PCR to amplify immunoglobulin variable (V) region genes and cloned those genes into phage expression vectors.
25 Kang et al., 1991, Proc. Natl. Acad. Sci. USA 88:4363-4366 created a phagemid vector that could be used to express the V and constant (C) regions of the heavy and light chains of an antibody specific for an antigen. The heavy and light chain V-C regions were engineered to combine in the
30 periplasm to produce an antibody-like molecule with a functional antigen binding site. Infection of cells harboring this phagemid with helper phage resulted in the incorporation of the antibody-like molecule on the surface of phage that carried the phagemid DNA. This allowed for identification and
35 enrichment of these phage by screening with the antigen. It was suggested that the enriched phage could be subject to mutation and further rounds of screening, leading to the

isolation of antibody-like molecules that were capable of even
stronger binding to the antigen.

Hoogenboom et al., 1991, Nucleic Acids Res. 19:4133-
4137 suggested that naïve antibody genes might be cloned into
5 phage display libraries. This would be followed by random
mutation of the cloned antibody genes to generate high
affinity variants.

In the prior art, peptide libraries have been
screened with receptors to identify receptor ligand-like
10 peptides, but peptide libraries have not been considered
useful for identifying such ligand-binding peptides as those
that mimic receptors.

Bass et al., 1990, Proteins: Struct. Func. Genet.
8:309-314 fused human growth hormone (hGH) to the carboxy
15 terminus of the gene III protein of phage fd. This fusion
protein was built into a phagemid vector. When cells carrying
the phagemid were infected with a helper phage, about 10% of
the phage particles produced displayed the fusion protein on
their surfaces. These phage particles were enriched by
20 screening with hGH receptor-coated beads. It was suggested
that this system could be used to develop mutants of hGH with
altered receptor binding characteristics.

Lowman et al., 1991, Biochemistry 30:10832-10838
used an improved version of the system of Bass et al.
25 described above to select for mutant hGH proteins with
exceptionally high affinity for the hGH receptor. The authors
randomly mutagenized the hGH-pIII fusion proteins at sites
near the vicinity of 12 amino acids of hGH that had previously
been identified as being important in receptor binding.
30   Balass et al., 1993, Proc. Natl. Acad. Sci. USA
90:10638-10642 used a phage display library to isolate linear
peptides that mimicked a conformationally dependent epitope of
the nicotinic acetylcholine receptor. This was done by
screening the library with a monoclonal antibody specific for
35 the conformationally dependent epitope. The monoclonal
antibody used was thought to be specific to the acetylcholine

receptor's binding site for its natural ligand, acetylcholine.

Citation or identification of any reference herein
shall not be construed as an admission that such reference is
5 available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to abtides. As used
herein, the term "abtides" refers to peptides that mimic the
10 binding specificity of a larger molecule such as an antibody
or receptor. Abtides specifically bind to a ligand of
interest, in which the ligand is a specific binding partner of
the larger molecule (e.g. antibody or receptor). To identify
the abtides of the present invention, peptide libraries are
15 screened in a two-step process. The first screening step uses
an antibody (or antigen-binding derivative thereof) or
receptor (or ligand-binding derivative thereof) as a first
target ligand. This step identifies peptide sequences termed
"epitopes" or "mimetopes" which specifically bind the first
20 target ligand. In the case where an antibody or derivative
thereof is used as the first target ligand, a mimetope will
often resemble, either functionally in terms of its binding
capability and/or structurally in terms of its amino acid
sequence, the epitope recognized by the antibody used as the
25 first ligand. An epitope or mimetope is then used as a second
target·ligand in a second screening step to identify a peptide
sequence that specifically binds the epitope or mimetope.
Such peptides are known as "abtides." Surprisingly, it was
found by the current inventors and is demonstrated herein that
30 abtides possess binding specificities strikingly similar to
those possessed by the first target ligands (usually
antibodies or receptors) described above.

Abtides are useful since they mimic the binding
specificities of antibodies or receptors. Thus, they may be
35 used in many instances where antibodies or receptors may be
used. The present invention further relates to the use of
abtides in the place of antibodies in assays such as the many

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types of immunoassays known in the art. Abtides may take the place of antibodies in such assays as, for example, enzyme-linked immunosorbent assays (ELISAs) or sandwich immunoassays. The invention also provides abtide compositions for use in

5 therapy and diagnosis. In a specific example, abtides have been discovered and demonstrated to be useful in place of antibodies in enzyme-linked immunosorbent assays and in *in vivo* localization to prostate carcinoma in a xenograft model.

10   The use of abtides has many potential advantages over the use of antibodies or receptors: the smaller size of abtides allows their easier production at lower cost, reduced immunogenicity, and may facilitate their *in vivo* delivery if such is desired; biological reactions and functions mediated

15 by constant domains of antibodies, and cross-linking of antibodies/receptors and resulting biological effects can be avoided if desired.

4.   FIGURE LEGENDS

20   The present invention may be understood more fully by reference to the following detailed description of the invention, examples of specific embodiments of the invention and the appended figures in which:

Figure 1 shows in schematic diagram a general method

25 for identifying an abtide by a two-step screening process. See Section 5.3 for a discussion of this method.

Figure 2 shows the binding of biotinylated monoclonal antibody 7E11-C5 to immobilized mimetope peptide 7E11-9.5. See Section 6.1.2.2 for details.

30   Figure 3 shows similarities in the amino acid sequences of the CDR2L and CDR3L regions of monoclonal antibody 7E11-C5 and the 7E11-C5 abtides of Table 2. The number of amino acids in the abtides that are similar to the CDRs is indicated in parentheses, along with the percent

35 homology. Dashes indicate gaps which have been added to improve the homology. In the case of clones 13 and 16, the homology with CDR2L was greatest if the sequence of CDR2L was

- 10 -

reversed. The sequence shown for clone 14 is SEQ ID NO: 1; the sequence shown for clone 17 is SEQ ID NO: 2; The sequence shown for clone 15 is SEQ ID NO: 3; the sequence shown for clone 13 is SEQ ID NO: ·4; the sequence shown for clone 16 is

5 SEQ ID NO: 5; the sequence shown for CDR3L is SEQ ID NO: 6; the sequence shown for CDR2L is SEQ ID NO: 7; the sequence shown for CDR2L(rev) is SEQ ID NO: 8.

Figure 4 shows binding of abtides to the 7E11-9.5 mimetope peptide in a dot blot assay as described in Section

10 6.1.2.1. Numbers along the left side of the figure refer to the 7E11-C5 abtide that was spotted in the indicated position. The number 351 refers to the monoclonal antibody 7E11-C5, used as a positive control. The numbers along the top of the figure refer to the various dilutions of the abtide or the

15 monoclonal antibody that were used.

Figure 5 shows the binding of biotinylated mimetopes to immobilized abtides. □ represents binding of mimetope peptide Biotin-LYANPGMYSRLHSPA-NH$_2$ to 7E11-C5 abtide clone 14; O represents binding of mimetope peptide Biotin-

20 LYANPGMYSRLHSPA-NH$_2$ to 7E11-C5 abtide clone 17; ◇ represents binding of mimetope peptide Biotin-GMYSRLH-NH$_2$ to 7E11-C5 abtide clone 14; Δ represents binding of mimetope peptide Biotin-GMYSRLH-NH$_2$ to 7E11-C5 abtide clone 17. See Section 6.1.2.2 for details.

25 Figure 6 shows the capture of an antigen from a lysate of LNCaP tumor cells by the monoclonal antibody 7E11-C5 and the 7E11-C5 abtide clone 14. See Section 6.1.3 for details.

Figure 7 shows the biodistribution of abtide clone

30 14-DPTA-^{111}In in SCID mice bearing human prostate carcinoma LNCaP xenograft tumors 2 hours (▨, bar on the left for each pair of bars) or 4 hours (▨, bar on the right for each pair of bars) post-injection of 2 μg of peptide, specific activity 32 μCi/μg. See Section 6.1.4 for details.

35 Figure 8 shows the biodistribution of abtide clone 17-DPTA-^{111}-In in four SCID mice bearing human prostate LNCaP carcinoma xenograft tumors 2 hours (▨, leftmost bar for

each group of four bars, mouse 1; ⬛, second bar from left
for each group of four bars, mouse 6) or five hours (⬛ ,
third bar from left for each group of four bars, mouse 2;
⬛ , rightmost bar for each group of four bars, mouse 4)

5 post-injection of 0.02 μg of peptide, specific activity 2.4
μCi/ng. See Section 6.1.4 for details.

    Figure 9 shows the biodistribution of [111]-In labeled
control irrelevant peptide in SCID mice bearing human prostate
carcinoma LNCaP xenograft tumors 2 hours (⬛ , leftmost bar

10 for each group of five bars; ⬛ , second bar from left for
each group of five bars) or 5 hours (⬛ , third bar from left
for each group of five bars; ⬛ , fourth bar from left for
each group of five bars; ⬛ , rightmost bar for each group of
five bars) post-injection of 1.5 μg of peptide, specific

15 activity 30 μCi/μg. See Section 6.1.4 for details.

    Figure 10 schematically illustrates the construction
of the R26 TSAR library. The R26 expression library was
constructed essentially as described for the TSAR-9 library
that is described in PCT publication WO 94/18318, dated August

20 18, 1994, except for the modifications depicted in Figure 10.
The oligonucleotide assembly process depicted in Figure 10
results in expression of peptides with the following amino
acid sequence:

S(S/R)$X_{12}\pi$AδX_{12}SR (SEQ ID NO: 89), where π = S, P, T or A; and

25 δ = V, A, D, E OR G.

    Figure 11 schematically illustrates the construction
of the D38 TSAR library. The D38 expression library was
constructed essentially as described for the TSAR-9 library
that is described in PCT publication WO 94/18318, dated August

30 18, 1994, except for the modifications depicted in Figure 11.

    Figure 12 schematically illustrates the construction
of the DC43 TSAR library. The DC43 expression library was
constructed essentially as described for the TSAR-9 library
that is described in PCT publication WO 94/18318, dated August

35 18, 1994, except for the modifications depicted in Figure 12.

    Figure 13 schematically illustrates the
oligonucleotides used to construct the polymorphic epithelial

mucin (PEM) abtide saturation mutagenesis TSAR library (See
Section 6.2.2).

5. DETAILED DESCRIPTION OF THE INVENTION

5   The present invention relates generally to abtides.
As used herein, the term "abtides" refers to peptides that
mimic the binding specificity of a larger molecule such as an
antibody or receptor. Abtides specifically bind to a ligand
of interest, in which the ligand is a specific binding partner
10 of the mimicked larger molecule (*e.g.* antibody or receptor).
To identify the abtides of the present invention, peptide
libraries are typically screened in a two-step process (see
Figure 1). The first screening step uses an antibody (or
antigen-binding derivative thereof) or receptor (or ligand-
15 binding derivative thereof) as a first target ligand. This
step identifies peptide sequences termed "epitopes" or
"mimetopes" which specifically bind the first target ligand.
If the first screening step uses an antibody and the peptide
identified contains the amino acid sequence of the natural
20 antigen that is responsible for the specific binding of the
antigen to the antibody, then the identified peptide is said
to be an epitope; if the identified peptide does not contain
the sequence of the natural antigen, then the identified
peptide is said to be a mimetope. In the case where an
25 antibody or derivative thereof is used as the first target
ligand, a mimetope will often resemble, either functionally in
terms of its binding capability and/or structurally in terms
of its amino acid sequence, the epitope recognized by the
antibody used as the first ligand.

30   A mimetope is then used as a second target ligand in
a second screening step to identify a peptide sequence that
specifically binds the epitope or mimetope. Such peptides are
known as "abtides." Abtides possess binding specificities
similar to those possessed by the first target ligands
35 (usually antibodies or receptors) described above.

          In a specific embodiment, the antibody or derivative
thereof used in the first screening step recognizes a tumor

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antigen, preferably a human tumor antigen, most preferably of
a malignant tumor.

The present invention provides a method to
successfully screen against very small peptide or protein
5 targets, *e.g.* 5 to 40 amino acids, preferably 10 to 20 amino
acids. To date, screening against such targets has not been
successful. The methods of the present invention increase the
likelihood that the abtide obtained will bind its target in a
complex or structurally dependent fashion.

10 Abtides are useful since they mimic the binding
specificities of antibodies or receptors. Thus, they may be
used in many instances where antibodies or receptors may be
used. The present invention further relates to the use of
abtides in the place of antibodies in assays such as the many
15 types of immunoassays known in the art. Abtides may take the
place of antibodies in such assays as, for example, enzyme-
linked immunosorbent assays (ELISAs) or sandwich immunoassays.
The invention also provides abtide compositions for use in
therapy and diagnosis of disease. In a specific example,
20 abtides have been produced and demonstrated to be useful in
place of antibodies in enzyme-linked immunosorbent assays and
in *in vivo* localization to prostate carcinoma in xenograft
models.

The use of abtides has many potential advantages
25 over the use of antibodies or receptors: the smaller size of
abtides allows their easier production at lower cost, reduced
immunogenicity, and facilitates their *in vivo* delivery if such
is desired; biological reactions and functions mediated by
constant domains of antibodies, and cross-linking of
30 antibodies/receptors and resulting biological effects can be
avoided if desired.

5.1. PEPTIDE LIBRARIES FOR USE IN IDENTIFYING ABTIDES

The abtides of the present invention can be
35 identified from a chemically synthesized peptide library or a
biologically expressed peptide library. If a biological
peptide expression library is used, the nucleic acid which

encodes the peptide which binds to the ligand of choice can be recovered, and then sequenced to determine its nucleotide sequence and hence deduce the amino acid sequence that mediates binding. Alternatively, the amino acid sequence of

5 an appropriate binding domain can be determined by direct determination of the amino acid sequence of a peptide selected from a peptide library containing chemically synthesized peptides. In a less preferred aspect, direct amino acid sequencing of a binding peptide selected from a biological

10 peptide expression library can also be performed.

In a preferred embodiment of the present invention, the abtides are advantageously identified from random peptide libraries. Typically, random peptide libraries will be encoded by synthetic oligonucleotides with a plurality of

15 variant nucleotide positions having the potential to encode all 20 naturally occurring amino acids. The sequence of amino acids encoded by the variant nucleotides is unpredictable and substantially random. The terms "unpredicted", "unpredictable" and "substantially random" are used

20 interchangeably with respect to the amino acids encoded and are intended to mean that the variant nucleotides at any given position are such that it cannot be predicted which of the 20 naturally occurring amino acids will appear at that position. These variant nucleotides are the product of random chemical

25 synthesis. The biological random peptide libraries envisioned for use include those in which a bias has been introduced into the random sequence, e.g., to disfavor stop codon usage.

5.1.1. CHEMICALLY SYNTHESIZED PEPTIDE LIBRARIES

30 The peptide libraries used in the present invention may be libraries that are chemically synthesized *in vitro*. Examples of such libraries are given in Fodor et al., 1991, Science 251:767-773, which describes the synthesis of a known array of short peptides on an individual microscopic slide;

35 Houghten et al., 1991, Nature 354:84-86, which describes mixtures of free hexapeptides in which the first and second residues in each peptide were individually and specifically

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defined. Lam et al., 1991, Nature 354:82-84, which describes a split synthesis scheme; Medynski, 1994, Bio/Technology 12:709-710, describes split synthesis and T-bag synthesis methods as well. See also Gallop et al., 1994, J. Medicinal

5 Chemistry 37:1233-1251.

PCT publication WO 91/05058, dated April 18, 1991, is directed to random libraries containing semi-random nucleotide sequences. The semi-random nucleotide sequences are transcribed *in vitro* under conditions such that polysomes

10 are produced. The polysomes are screened for binding to a substance of interest. Those polysomes that bind to the substance of interest are recovered. The RNA from those polysomes is used to construct cDNA, which is expressed to produce polypeptides.

15 Screening to identify peptides which bind to a ligand of choice can be carried out by methods well known in the art.

In a specific embodiment, the total number of unpredictable amino acids in the peptides of the chemical

20 library used for screening is greater than or equal to 5 and less than or equal to 25; in other embodiments the total is in the range of 5-15 or 5-10 amino acids, preferably contiguous amino acids.

While a binding domain can be identified from

25 chemically synthesized peptide libraries, such a domain would be small (i.e. less than 10 amino acids, and most probably 5-6 amino acids, in length). Therefore, use of a chemically synthesized peptide library is less preferred for the second screening step involved in isolating abtides than is use in

30 the second screening step of biological peptide libraries containing unpredictable sequences of greater length, described below.

5.1.2. <u>BIOLOGICAL PEPTIDE LIBRARIES</u>

35 In another embodiment, biological peptide libraries are used to identify abtides. Many suitable biological peptide libraries are known in the art and can be used.

According to this second approach, involving recombinant DNA techniques, peptides have been expressed in biological systems as either soluble fusion proteins or viral capsid fusion proteins:

5   A number of peptide libraries according to this approach have used the M13 phage. Although the N-terminus of the viral capsid protein, protein III (pIII), has been shown to be necessary for viral infection, the extreme N-terminus of the mature protein does tolerate alterations such as

10 insertions.   Accordingly, various peptide libraries, in which the diverse peptides are expressed as pIII fusion proteins, are known in the art; these libraries can be used to identify abtides. Examples of such libraries are described below.

15   Scott and Smith, 1990, Science 249:386-390 describe construction and expression of an "epitope library" of hexapeptides on the surface of M13. The library was made by inserting a 33 base pair Bgl⁻I digested oligonucleotide sequence into an Sfi I digested phage fd-tet, *i.e.*, fUSE5 RF.

20 The 33 base pair fragment contains a random or "degenerate" coding sequence $(NNK)_6$ where N represents G, A, T or C and K represents G or T. The authors stated that the library consisted of 2×10^8 recombinants expressing 4×10^7 different hexapeptides; theoretically, this library expressed 69% of the

25 6.4×10^7 possible peptides (20^6). Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87: 6378-6382 also described a somewhat similar library of hexapeptides expressed as pIII gene fusions of M13 fd phage. PCT publication WO 91/19818 dated December 26, 1991 by Dower and Cwirla describes a similar library of

30 pentameric to octameric random amino acid sequences.

Devlin et al., 1990, Science, 249:404-406, describes a peptide library of about 15 residues generated using an (NNS) coding scheme for oligonucleotide synthesis in which S is G or C.

35   Christian and colleagues have described a phage display library, expressing decapeptides (Christian et al., 1992, J. Mol. Biol. 227:711-718). The starting DNA was

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generated by means of an oligonucleotide comprising the degenerate codons $[NN(G/T)]_{10}$ with a self-complementary 3' terminus. This sequence, in forming a hairpin, creates a self-priming replication site which could be used by T4 DNA

5 polymerase to generate the complementary strand. The double-stranded DNA was cleaved at the SfiI sites at the 5' terminus and hairpin for cloning into the fUSE5 vector described by Scott and Smith, *supra*.

Lenstra, 1992, J. Immunol. Meth. 152:149-157

10 describes construction of a library by a laborious process encompassing annealing oligonucleotides of about 17 or 23 degenerate bases with an 8 nucleotide long palindromic sequence at their 3' ends. This resulted in the expression of random hexa- or octa-peptides as fusion proteins with the β-

15 galactosidase protein in a bacterial expression vector. The DNA was then converted into a double-stranded form with Klenow DNA polymerase, blunt-end ligated into a vector, and then released as Hind III fragments. These fragments were then cloned into an expression vector at the sequence encoding the

20 C-terminus of a truncated β-galactosidase to generate 10^7 recombinants.

Other biological peptide libraries which can be used include those described in U.S. Patent No. 5,270,170 dated December 14, 1993 and PCT Publication No. WO 91/19818 dated

25 December 26, 1991. Also suitable are those in U.S. Patent No. 5,096,815, U.S. Patent No. 5,198,346, and U.S. Patent No. 5,223,409, all to Ladner et al.

The biological peptide libraries discussed above are meant to be illustrative and not limiting. It will be

30 recognized by one of skill in the art that many other biological peptide libraries disclosed in various publications may be suitable for use in the practice of the present invention.

The protein pVIII is a major M13 viral capsid

35 protein, which can also serve as a site for expressing peptides on the surface of M13 viral particles, in the construction of random peptide libraries.

While it would be understood by one skilled in the art that as few as 5 amino acids can constitute a binding domain, the average functional domain within a natural protein is considered to be about 40 amino acids. Thus, the random

5 peptide libraries from which the abtides of the present invention are preferably identified encode peptides having in the range of 5 to 200 total variant amino acids. Although it is contemplated that biologically expressed random peptide libraries displaying short random inserts (i.e. less than 20

10 amino acids in length) could be used to identify abtides of the invention, the most preferred biologically expressed random peptide libraries for use in the invention are those in which the displayed peptide has 20 or greater unpredictable amino acids *i.e.* preferably in the range of 20 to 100, and

15 most preferably 20 to 50 amino acids, as exemplified by the TSAR libraries described in PCT publication WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated August 18, 1994.

To identify abtides, particularly in the second

20 screening step, the invention preferably uses libraries of greater complexity than are commonly employed in the art. The conventional teaching in the random peptide library art is that the length of inserted oligonucleotides should be kept short, encoding preferably fewer than 15 and most preferably

25 about 6-8 amino acids. However, not only can libraries encoding more than about 20 amino acids be constructed, but such libraries can be advantageously screened to identify peptides having binding specificity for a variety of ligands. Such libraries with longer length inserts are exemplified by

30 the TSAR libraries, described in PCT publication WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated August 18, 1994.

These PCT publications disclose that the use of libraries composed of longer length oligonucleotides has many

35 advantages.

Libraries composed of longer length oligonucleotides afford the ability to identify peptides in which a short

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sequence of amino acids is common to or shared by a number of peptides binding a given ligand, *i.e.*, library members having shared binding motifs. The use of longer length libraries also affords the ability to identify peptides which do not
5 have any shared sequences with other peptides but which nevertheless have binding specificity for the same ligand.

When screened by the method of the present invention, libraries having large inserted oligonucleotide sequences provide the opportunity to identify or map binding
10 sites which encompass not only a few contiguous amino acid residues, *i.e.*, simple binding sites, but also those which encompass discontinuous amino acids, *i.e.*, complex binding sites, and may afford the complex binding characteristic of antibodies and receptor-like molecules.

15 Additionally, the large size of the inserted synthesized oligonucleotides of certain libraries provides the opportunity for the development of secondary and/or tertiary structure in the potential binding peptides and in sequences flanking the actual binding site in the binding domain.
20 Secondary and tertiary structure often significantly affect the ability of a sequence to mediate binding, as well as the strength and specificity of any binding which occurs. Such complex structural effects are not possible when only small length oligonucleotides are used in libraries. It may be that
25 secondary and tertiary structures are especially important in the identification of abtides since abtides mimic the binding of large molecules such as antibodies. It is well known that the antigen binding properties of antibodies depend in most instances upon several different regions of the heavy chain
30 (complementarity determining regions) and upon regions contributed by the light chain as well.

Therefore, it is contemplated that the most preferred binding domains for identifying the abtides of the present invention will be those from biologically expressed
35 random peptide libraries in which the displayed peptide is 20 or greater amino acids in length. Examples of such random peptide libraries are the TSAR libraries, described in in PCT

publication WO 91/12328, dated August 22, 1991, and PCT
publication WO 94/18318, dated August 18, 1994.

In one embodiment, the library utilized in the
present invention is a linear, non-constrained library. As
5 would be understood by one in the art having considered the
present disclosure, in another specific embodiment,
"constrained", "structured" or "semi-rigid" random peptide
libraries could also be used in the present methods to
identify abtides. Typically, these libraries express peptides
10 that are substantially random but contain a small percentage
of fixed residues within or flanking the random sequences that
have the result of conferring structure or some degree of
conformational rigidity to the peptide. In a semirigid
peptide library, the plurality of synthetic oligonucleotides
15 express peptides that are each able to adopt only one or a
small number of different conformations that are constrained
by the positioning of codons encoding certain structure
conferring amino acids in or~flanking the synthesized variant
or unpredicted oligonucleotides. Unlike linear, unconstrained
20 libraries in which the plurality of proteins expressed
potentially adopt thousands of short-lived different
conformations, in a semirigid peptide library, the plurality
of proteins expressed can adopt only a single or a small
number of conformations. Such libraries are exemplified by the
25 TSAR-13 and TSAR-14 libraries described in PCT publication WO
94/18318, dated August 18, 1994; by a library of random 6
amino acid sequences, each flanked by invariant cysteine
residues (O'Neil et al., 1992, Proteins 14:509-515); and by
those libraries disclosed in PCT Publication No. WO 94/11496,
30 dated May 26, 1994 (Huse).

In a preferred embodiment, a biological peptide
library that is a random peptide "TSAR" library is screened to
identify an abtide. TSARs is an acronym for "Totally
Synthetic Affinity Reagents" as described in PCT publication
35 WO 91/12328, dated August 22, 1991, and PCT publication WO
94/18318, dated August 18, 1994. TSAR libraries, their
construction and use, and specific examples of TSAR libraries,

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are described in detail in those PCT publications. Nucleic acids encoding TSARs or a TSAR portion which mediates binding to the ligand used for screening can be used to identify the abtides of the present·invention.

5   A TSAR may be a heterofunctional fusion protein, said fusion protein comprising (a) a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of

10 unpredictable nucleotides is greater than or equal to about 60 and less than or equal to about 600, and optionally, (b) an effector domain encoded by an oligonucleotide sequence which is a protein or peptide that enhances expression or detection of the binding domain.

15   Alternatively, a TSAR may be a heterofunctional fusion protein as described above but in which the contiguous sequences are flanked by invariant residues designed to encode amino acids that confer a desired structure to the binding domain of the expressed heterofunctional fusion protein.

20   In addition to TSAR libraries, other libraries for use in the present invention may be those wherein the library is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide

25 comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

30

5.2.   ABTIDES

An abtide is typically a peptide that mimics, with respect to binding specificity, and possibly other

35 characteristics (e.g., binding affinity, sequence, etc.) a large molecule such as an antibody or receptor. However, an

abtide is generally much smaller than an antibody or receptor. An abtide is generally a peptide of about 5 to 200 amino acids. Preferably, an abtide is a peptide of about 10 to 100 amino acids. Most preferably, an abtide is a peptide of about

5 20 to 50 amino acids. In addition to the amino acid sequences which are responsible for the abtide's specific binding properties, an abtide may be linked to additional amino acid sequences or additional non-amino acid sequences. Such additional sequences may aid in the identification or

10 isolation of the abtide. Or, they may aid in the biodistribution, stability, or diagnostic or therapeutic effectiveness of the abtide when the abtide is used diagnostically or therapeutically.

The abtides may be linked to a variety of non-

15 peptide moieties. Such moieties might include toxins; drugs; polysaccharides; nucleotides; oligonucleotides; labels such as radioactive substances (e.g. ^{111}In, ^{125}I, ^{131}I, ^{99m}Tc, ^{212}B, ^{90}Y, ^{186}Rh); biotin; fluorescent tags; imaging reagents (e.g. those described in U.S. Patent No. 4,741,900 and U.S. Patent No.

20 5,326,856); hydrocarbon linkers (e.g., an alkyl group or derivative thereof) conjugated to a moiety providing for attachment to a solid substratum, or to a moiety providing for easy separation (e.g., a hapten recognized by an antibody bound to a magnetic bead), etc. Linkage of the peptide to the

25 non-peptide moiety may be by any of several well-known methods in the art.

In addition, in an embodiment in which an abtide has a free amino- or carboxy- terminus, such termini can be modified by known methods, e.g., to provide greater resistance

30 to degradation, greater cell permeability, greater solubility, etc., e.g., by acetylation, biotinylation, fatty acylation, etc. at the amino-terminus; amidation at the carboxy-terminus; or the abtide can be stabilized by inclusion of D amino acids, nonnatural amino acids or glycosyl amino acids, etc.

35 The abtides of the invention are preferably made by commonly known methods of chemical synthesis, e.g., as

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described by way of example in Section 5.6 and its
subsections.

    Alternatively, abtides (or portions thereof) can be
obtained by recombinant expression of a nucleic acid encoding

5 the desired abtide in an appropriate host, by well-known
methods.

    Occasionally, it may happen that not all of the
amino acids in the identified peptide will be necessary for
the binding function of an abtide. Where it is desired to

10 decrease the size of the abtide, methods can be used to
identify portions of the determined synthetic amino acid or
nucleotide sequences which respectively mediate binding, or
encode the sequences which mediate binding, as described in
Section 5.4 below.

15

5.3. SCREENING OF PEPTIDE LIBRARIES TO IDENTIFY ABTIDES

    The process of identifying abtides from a peptide
library comprises two distinct screening steps. The first

20 step is designed to identify epitopes or ligands with binding
specificity for the larger molecule of interest, *e.g.* antigen
mimics, receptor-ligand mimics, and the like. In that first
step, a peptide library is screened with a ligand that
possesses a specific, often complex, binding site of interest.

25 Those peptides in the library that are specific binding
partners of the ligand bind to the ligand and are readily
recoverable because of this specific binding. An example of a
ligand suitable for use in this first screening step would be
an antibody, inherently possessing an antigen binding site, or

30 an antigen-binding derivative thereof. Another example would
be a receptor *e.g.* the epidermal growth factor receptor or the
platelet derived growth factor receptor. These particular
receptors possess specific binding sites for epidermal growth
factor and platelet derived growth factor, respectively.

35 Other receptors are known in the art and are also potential
ligands, for example, the estrogen receptor, the various

acetylcholine receptors, the human growth hormone receptor, etc.

Molecules comprising those peptide sequences in the library that are identified by the first screening step will
5 be referred to herein as epitopes or mimetopes. For example, in the case where the first ligand is an antibody, the peptides in the library that are identified as specific binding partners of the antibody would be known as antigen epitopes or mimetopes.

10 The peptides that are isolated in the first screening step are then preferably analyzed, as, for example, by DNA sequencing of the binding domain of the phage that encode the peptides if the library used was a phage library. The DNA sequence of the binding domain encodes the amino acid
15 sequence of the epitope or mimetope peptide. Due to the known relationship between DNA sequences and their encoded amino acid sequences, obtaining the DNA sequence of the epitope or mimetope allows the determination of the amino acid sequence of the epitope or mimetope. Alternatively, if the library
20 used was a chemical library, direct amino acid sequencing of the peptide epitope or mimetope can be carried out by well known methods in the art.

In a specific embodiment, sequences of different peptide mimetopes identified in the first screening step can
25 be compared to determine a consensus mimetope sequence.

Once the amino acid sequence of the epitope or mimetope is known (or a portion thereof, which mediates binding), a molecule, preferably a peptide, is produced comprising that amino acid sequence which mediates binding.
30 This peptide may be synthesized chemically, or, alternatively, may be produced by methods involving recombinant DNA. This peptide may contain only the amino acids of the epitope or mimetope or, preferably, it may contain additional amino acids or non-amino acid moieties to aid in identifying or recovering
35 the epitope or mimetope peptide and any new peptide binders found in the subsequent screening step discussed below.

In the second screening step, the epitope or mimetope that was identified in the first screening step is used as a ligand for the second screening step. The second screening step identifies peptides with binding specificity

5 for the epitope or mimetope and that surprisingly mimic the binding specificity of the antibody or receptor that was used as ligand in the first screening step. In other words, the second screening step yields peptides with antibody or receptor-like binding activity for antigens or receptor

10 ligands that are known as abtides.

Figure 1 is a schematic representation of an exemplary two-step screening process used to identify abtides.

In a particular embodiment of the invention, it may be that the epitope to which an antibody specifically binds is

15 known. For example, the monoclonal antibody SM-3 that specifically binds the polymorphic epithelial mucin (PEM) found on human breast carcinoma cells has been shown to be specific for the epitope defined by the amino acid sequence VTSAPDTRPAPGSTAPPAHGVTSAPDTR (SEQ ID NO: 9) (Burchell et al.,

20 1989, Int. J. Cancer 44:691-696). In such cases, the first screening step described above may be dispensed with. A peptide comprising the sequence of the epitope for which the antibody is specific can be synthesized and used in the second screening step described above in order to identify abtides of

25 the antibody.

It may also be that the portion of a "receptor-ligand" (*i.e.*, a ligand which specifically binds to a receptor) to which a receptor specifically binds is known. For example, it has been shown that granulocyte/macrophage

30 colony stimulating factor (GM-CSF) binds to the GM-CSF receptor through amino acids 88-121 (HCPPTPETSCATQTITFESFKENLKDFLLVIPFDC [SEQ ID NO: 22]) of GM-CSF. It should be possible to synthesize a peptide corresponding to the portion of the receptor-ligand that has

35 been shown to be responsible for specific binding to the receptor and to use such a peptide in the second screening

step of the methods of the present invention in order to identify an abtide of the receptor.

As used in the present invention, a ligand is a substance for which it is desired to isolate a specific 5 binding partner from a peptide library. A ligand can function as a lock, *i.e.*, a large polypeptide or protein analogous to a lock into which a smaller specific binding partner fits as a key; or a ligand can function as a key which fits into and specifically binds a larger binding partner or lock.

10 In this invention, an epitope or mimetope is typically a peptide that acts as a key; it is identified by screening a peptide library for peptides that fit into and bind the specific binding site of a larger molecule which acts as a lock, *e.g.* antibody or receptor. If the larger molecule 15 is an antibody and the peptide identified contains the portion of the amino acid sequence of the natural antigen that is responsible for the specific binding of the antigen to the antibody, then the identified peptide is said to be an epitope; if the identified peptide does not contain the 20 sequence of the natural antigen, then the identified peptide is said to be a mimetope.

In a specific embodiment, a mimetope is identified by screening a peptide library with an antibody or antibody fragment. Mimetopes thus identified functionally mimic the 25 antigen to which the antibody binds in that the mimetopes also specifically bind with the antibody. In some cases, if the antigen is a protein or polypeptide, the mimetopes may share amino acid sequence motifs with the antigen. In another embodiment, a mimetope is identified by screening a peptide 30 library with a receptor or receptor fragment. Mimetopes thus identified functionally mimic the natural ligand of the receptor.

The peptide libraries that are used in the first and second screening steps may be the same or different. In one 35 embodiment, a peptide library containing small random inserts (from about 6 to about 15 amino acids) is used in the first screening step.

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In the second screening step, it may be desirable to use a larger peptide library. Such larger libraries preferably express peptides of about 20 to 200 random amino acids. Examples of such larger libraries are the TSAR

5 libraries described in PCT publication WO 91/12328, dated August 22, 1991, and in PCT Publication WO 94/18318, dated August 18, 1994.

Biological or chemically synthesized peptide libraries can be used in either the first or second

10 screenings. The peptide libraries used in the present invention may have a plurality of residues that are random, *i.e.* residues for which the amino acid occupying that residue cannot be predicted in advance. Such libraries are said to be random peptide libraries.

15 A preferred method for identifying abtides comprises screening a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising (a) a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which

20 the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600, and optionally, (b) an effector domain encoded by an oligonucleotide sequence which

25 is a protein or peptide that enhances expression or detection of the binding domain. Screening is done by contacting the plurality of heterofunctional fusion proteins with a ligand under conditions conducive to ligand binding and then isolating the fusion proteins which bind to the ligand. The

30 methods of the invention further preferably comprise determining the nucleotide sequence encoding the binding domain of the heterofunctional fusion protein identified to determine the DNA sequence that encodes the binding domain and simultaneously to deduce the amino acid sequence of the

35 mimetope used in the second screen. Nucleotide sequence analysis can be carried out by any method known in the art, including but not limited to the method of Maxam and Gilbert

(1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method
(Sanger et al., 1977, Proc. Natl, Acad. Sci. U.S.A. 74:5463),
the use of T7 DNA polymerase (Tabor and Richardson, U.S.
Patent No. 4,795,699; Sequenase™, U.S. Biochemical Corp.), or
5 Taq polymerase, or use of an automated DNA sequenator (e.g.,
Applied Biosystems, Foster City, CA).

    Alternatively, the libraries used to screen for
mimetopes and abtides of the invention have unpredictable
nucleotides arranged in one or more contiguous sequences that
10 are flanked by invariant residues designed to encode amino
acids that confer a desired structure to the binding domain of
the expressed heterofunctional fusion protein.

    Once a suitable peptide library has been constructed
(or otherwise obtained), the library is screened to identify
15 peptides having binding affinity for a ligand of choice.
Screening the libraries can be accomplished by any of a
variety of methods known to those of skill in the art. See,
e.g., the following references, which disclose screening of
peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med.
20 Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390;
Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et
al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al.,
1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-
580; Bock et al., 1992, Nature 355:564-566; Tuerk et al.,
25 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et
al., 1992, Nature 355:850-852; U.S. Patent No. 5,096,815,
U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all
to Ladner et al.; and Rebar and Pabo, 1993, Science 263:671-
673. See also PCT publication WO 94/18318, dated August 18,
30 1994.

    If the libraries are expressed as fusion proteins
with a cell surface molecule, then screening is advantageously
achieved by contacting the vectors with an immobilized target
ligand and harvesting those vectors that bind to said ligand.
35 Such useful screening methods, designated "panning" techniques
are described in Fowlkes et al., 1992, BioTechniques 13:422-
427. In panning methods useful to screen the libraries, the

target ligand can be immobilized on plates, beads, such as magnetic beads, sepharose, etc., or on beads used in columns. In particular embodiments, the immobilized target ligand can be "tagged", *e.g.*, using such as biotin, 2-fluorochrome, *e.g.*

5 for FACS sorting.

In one embodiment, presented by way of example but not limitation, screening a library of phage expressing random peptides on phage and phagemid vectors can be achieved by using magnetic beads as described in PCT publication WO

10 94/18318, dated August 18, 1994.

Alternatively, as yet another non-limiting example, screening a library of phage expressing random peptides can be achieved by panning using microtiter plates. In a preferred method for recovering the phage bound to the wells of the

15 microtiter plates, a pH change is used.

By way of another example, the libraries expressing random peptides as a surface protein of either a vector or a host cell, *e.g.*, phage or bacterial cell, can be screened by passing a solution of the library over a column of a ligand

20 immobilized to a solid matrix, such as sepharose, silica, etc., and recovering those phage that bind to the column after extensive washing and elution.

By way of yet another example, weak binding library members can be isolated based on retarded chromatographic

25 properties. According to one mode of this embodiment for screening, fractions are collected as they come off the column, saving the trailing fractions (*i.e.*, those members that are retarded in mobility relative to the peak fraction are saved). These members are then concentrated and passed

30 over the column a second time, again saving the retarded fractions. Through successive rounds of chromatography, it is possible to isolate those that have some affinity, albeit weak, to the immobilized ligand. These library members are retarded in their mobility because of the millions of possible

35 ligand interactions as the member passes down the column. In addition, this methodology selects those members that have

modest affinity to the target, and which also have a rapid dissociation time.

If desired, the oligonucleotides encoding the binding domain selected in this manner can be mutagenized, expressed and rechromatographed (or screened by another method) to discover improved binding activity. In particular, saturation mutagenesis can be carried out using synthetic oligonucleotides synthesized from "doped" nucleotide reservoirs. The doping is carried out such that the original peptide sequence is represented only once in 10^6 unique clones of the mutagenized oligonucleotide. The assembled oligonucleotides are cloned into a parental TSAR vector. Preferably, the vector is m663 (Fowlkes et al., 1992, BioTechniques 13:422-427). m663 is able to make blue plaques when grown in *E. coli* stain JM101 or DH5αF'. A library of greater than 10^6 is preferred; however a library of 10^5 is sufficient to isolate TSAR phage displaying peptide domains with increased selectivity for binding to the target ligand.

According to another alternative method, screening a library of can be achieved using a method comprising an "enrichment" step and a filter lift step as follows.

Random peptides from an expressed library capable of binding to a given ligand ("positives") are initially enriched by one or two cycles of panning or affinity chromatography, as described above. The goal is to enrich the positives to a frequency of about $> 1/10^5$. Following enrichment, a filter lift assay is conducted. For example, approximately 1-2 x 10^5 phage, enriched for binders, are added to 500 μl of log phase *E. coli* and plated on a large LB-agarose plate with 0.7% agarose in broth. The agarose is allowed to solidify, and a nitrocellulose filter (e.g., 0.45 μ) is placed on the agarose surface. A series of registration marks is made with a sterile needle to allow re-alignment of the filter and plate following development as described below. Phage plaques are allowed to develop by overnight incubation at 37 °C (the presence of the filter does not inhibit this process). The filter is then removed from the plate with phage from each

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individual plaque adhered *in situ*. The filter is then exposed
to a solution of BSA or other blocking agent for 1-2 hours to
prevent non-specific binding of the ligand (or "probe").

The probe itself is labeled, for example, either by
5 biotinylation (using commercial NHS-biotin) or direct enzyme
labeling, *e.g.*, with horse radish peroxidase (HRP) or alkaline
phosphatase. Probes labeled in this manner are indefinitely
stable and can be re-used several times. The blocked filter
is exposed to a solution of probe for several hours to allow
10 the probe to bind *in situ* to any phage on the filter
displaying a peptide with significant affinity to the probe.
The filter is then washed to remove unbound probe, and then
developed by exposure to enzyme substrate solution (in the
case of directly labeled probe) or further exposed to a
15 solution of enzyme-labeled avidin (in the case of biotinylated
probe). In a preferred method, an HRP-labeled probe is
detected by ECL western blotting methods (Amersham, Arlington
Heights, IL), which involves using luminol in the presence of
phenol to yield enhanced chemiluminescence detectable by brief
20 exposure of film by autoradiography, in which the exposed
areas of film correspond to positive plaques on the original
plate. Where an enzyme substrate is used, positive phage
plaques are identified by localized deposition of colored
enzymatic cleavage product on the filter which corresponds to
25 plaques on the original plate. The developed filter or film,
as the case may be, is simply realigned with the plate using
the registration marks, and the "positive" plaques are cored
from the agarose to recover the phage. Because of the high
density of plaques on the original plate, it is usually
30 impossible to isolate a single plaque from the plate on the
first pass. Accordingly, phage recovered from the initial
core are re-plated at low density and the process is repeated
to allow isolation of individual plaques and hence single
clones of phage.

35 Successful screening experiments are optimally
conducted using 3 rounds of serial screening. The recovered
cells are then plated at a low density to yield isolated

colonies for individual analysis. The individual colonies are selected and used to inoculate LB culture medium containing ampicillin. After overnight culture at 37°C, the cultures are then spun down by centrifugation. Individual cell aliquots

5 are then retested for binding to the target ligand attached to the beads. Binding to other beads, having attached thereto a non-relevant ligand, can be used as a negative control.

One important aspect of screening the libraries is that of elution. For clarity of explanation, the following is

10 discussed in terms of TSAR expression by phage; however, it is readily understood that such discussion is applicable to any system where the random peptide is expressed on a surface fusion molecule. It is conceivable that the conditions that disrupt the peptide-target interactions during recovery of the

15 phage are specific for every given peptide sequence from a plurality of proteins expressed on phage. For example, certain interactions may be disrupted by acid pH's but not by basic pH's, and vice versa. Thus, it may be desirable to test a variety of elution conditions (including but not limited to

20 pH 2-3, pH 12-13, excess target in competition, detergents, mild protein denaturants, urea, varying temperature, light, presence or absence of metal ions, chelators, etc.) and compare the primary structures of the TSAR proteins expressed on the phage recovered for each set of conditions to determine

25 the appropriate elution conditions for each ligand/TSAR combination. Some of these elution conditions may be incompatible with phage infection because they are bactericidal and will need to be removed by dialysis (i.e., dialysis bag, Centricon/Amicon microconcentrators).

30 The ability of different expressed proteins to be eluted under different conditions may not only be due to the denaturation of the specific peptide region involved in binding to the target but also may be due to conformational changes in the flanking regions. These flanking sequences may

35 also be denatured in combination with the actual binding sequence; these flanking regions may also change their secondary or tertiary structure in response to exposure to the

elution conditions (*i.e.*, pH 2-3, pH 12-13, excess target in competition, detergents, mild protein denaturants, urea, heat, cold, light, metal ions, chelators, etc.) which in turn leads to the conformational deformation of the peptide responsible

5 for binding to the target.

According to another alternative method in which the TSARs contain a linker region between the binding domain and the effector domain, particular TSAR libraries can be prepared and screened by: (1) engineering a vector, preferably a phage

10 vector, so that a DNA sequence encodes a segment of Factor Xa (or Factor Xa protease cleavable peptide) and is present adjacent to the gene encoding the effector domain, *e.g.*, the pIII coat protein gene; (2) construct and assemble the double stranded synthetic oligonucleotides as described above and

15 insert into the engineered vector; (3) express the plurality of vectors in a suitable host to form a library of vectors; (4) screen for binding to an immobilized ligand; (5) wash away excess phage; and (6) treat the immobilized phage with Factor Xa protease. The particle will be uncoupled from the peptide-

20 ligand complex and can then be used to infect bacteria to regenerate the particle with its full-length pIII molecule for additional rounds of screening. This alternative embodiment advantageously allows the use of universally effective elution conditions and thus allows identification of phage expressing

25 TSARs that otherwise might not be recovered using other known methods for elution. To illustrate, using this embodiment, exceptionally tight binding TSARs could be recovered.   If desired, the oligonucleotides encoding the binding domain selected in this manner can be mutagenized, expressed and

30 rechromatographed (or screened by another method) to discover improved binding activity. In particular, saturation mutagenesis can be carried out using synthetic oligonucleotides synthesized from "doped" nucleotide reservoirs. The doping is carried out such that the original

35 peptide sequence is represented only once in 10^6 unique clones of the mutagenized oligonucleotide. The assembled oligonucleotides are cloned into a parental TSAR vector.

Preferably, the vector is m663 (Fowlkes et al., 1992, BioTechniques 13:422-427). m663 is able to make blue plaques when grown in *E. coli* stain JM101 or DH5αF'. A library of greater than 10^6 is preferred; however a library of 10^5 is

5 sufficient to isolate TSAR phage displaying peptide domains with increased selectivity for binding to the target ligand.

For the examples in Section 6 herein, a TSAR library is utilized; however, to those skilled in the art, it will be apparent that other peptide libraries may be used. An example

10 of a TSAR library is the TSAR-9 library disclosed in Kay et al., 1993, Gene 128:59-65. TSAR-9 constructs display a peptide of about 38 amino acids in length having 36 totally random positions.

15   5.3.1.   ANTIBODIES AND DERIVATIVES THEREOF
                FOR USE IN SCREENING

Antibodies can be produced which recognize an antigen of interest. Such antibodies can be polyclonal or monoclonal. Such antibodies may be used as ligands in the

20 first screening step of the present invention.

Various procedures known in the art may be used for the production of polyclonal antibodies to an antigen of interest. For the production of antibody, various host animals can be immunized by injection with an antigen of interest or

25 derivative thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface

30 active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

35   A monoclonal antibody to an antigen of interest can be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in

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culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256:495-497), and the more recent human B cell hybridoma technique (Kozbor et al., 1983, Immunology Today

5 4:72) and EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

The monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species)

10 monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody molecules

15 may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

A molecular clone of an antibody to an antigen of

20 interest can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or

25 antigen binding region thereof.

Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof, etc.

30 Antibody fragments which contain the idiotype or antigen binding region of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments

35 which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the 2 Fab or Fab fragments which can

be generated by treating the antibody molecule with papain and a reducing agent.

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5.4. IDENTIFICATION OF SYNTHETIC
SEQUENCES WHICH MEDIATE BINDING
</div>

5

When a peptide from a peptide library has been identified as an abtide or mimetope for a particular target ligand of interest according to the method of the invention (in either the first or second screening step), it may be

10 useful to determine what region(s) of the expressed peptide sequence is (are) responsible for binding to the target ligand. Such analysis can be conducted at two different levels, *i.e.*, the nucleotide sequence and amino acid sequence levels.

15 By molecular biological techniques it is possible to verify and further analyze a ligand binding peptide at the level of the oligonucleotides. First, the inserted oligonucleotides can be cleaved using appropriate restriction enzymes and religated into the original expression vector and

20 the expression product of such vector screened for ligand binding to identify the oligonucleotides that encode the binding region of the abtide or mimetope. Second, the oligonucleotides can be transferred into another vector, *e.g.*, from phage to phagemid. The newly expressed fusion proteins

25 should acquire the same binding activity if the domain is necessary and sufficient for binding to the ligand. This last approach also assesses whether or not flanking amino acid residues encoded by the original vector influence peptide binding in any fashion. Third, the oligonucleotides can be

30 synthesized, based on the nucleotide sequence determined for the phage in the library that encodes the binding peptide, amplified by cloning or PCR amplification using internal and flanking primers, cleaved into two pieces and cloned as two half-binding domain fragments. In the foregoing manner, the

35 inserted oligonucleotides are subdivided into two equal halves. If the peptide domain important for binding is small, then one recombinant clone would demonstrate binding and the

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other would not. If neither have binding, then either both are important or the essential portion of the domain spans the middle (which can be tested by expressing just the central region).

5   Alternatively, by synthesizing peptides corresponding to the deduced sequence of the abtide or mimetope, the binding domains can be analyzed. First, the entire peptide should be synthesized and assessed for binding to the target ligand to verify that the peptide is necessary

10 and sufficient for binding. Second, short peptide fragments, for example, overlapping 10-mers, can be synthesized, based on the amino acid sequence of the random peptide binding domain, and tested to identify those binding the ligand.

        In addition, in certain instances, linear motifs

15 (consensus motifs) may become apparent after comparing the primary structures of different binding peptides from the library having binding affinity for a target ligand. The contribution of these motifs to binding can be verified with synthesized peptides in competition experiments (i.e.,

20 determine the concentration of peptide capable of inhibiting 50% of the binding of the phage to its target; IC_{50}). Conversely, the motif or any region suspected to be important for binding can be removed from or mutated within the DNA encoding the random peptide insert and the altered displayed

25 peptide can be retested for binding.

        Furthermore, once the binding domain of a peptide has been identified, the binding characteristics of that peptide can be modified by varying the binding domain sequence to produce a related family of peptides with differing

30 properties for a specific ligand.

        Moreover, in a method of directed evolution, the identified peptides can be improved by additional rounds of random mutagenesis, selection, and amplification of the nucleotide sequences encoding the binding domains.

35 Mutagenesis can be accomplished by creating and cloning a new set of oligonucleotides that differ slightly from the parent sequence, e.g., by 1-10%. Selection and amplification are

achieved as described above. By way of example, to verify
that the isolated peptides have improved binding
characteristics, mutants and the parent phage, differing in
their <u>lacZ</u> expression, ·can be processed together during the
5 screening experiments. Alteration of the original blue-white
color ratios during the course of the screening experiment
will serve as a visual means to assess the successful
selection of enhanced binders. This process can go through
numerous cycles.
10

5.5. <u>USES OF ABTIDES</u>

5.5.1. <u>ASSAYS USING ABTIDES</u>

The abtides of the present invention possess binding
15 specificities that are similar to those of the ligands (e.g.
antibodies, receptors) that are used in the first screening
step of the process by which the abtides are identified.
Consequently, the abtides may̆ be used in many of the same
instances where the ligand of the first screening step might
20 be used. For example, if the ligand used in the first
screening step is an antibody, the abtide that is identified
after the second screening step will bind specifically to the
same antigen to which the antibody specifically binds.
Therefore, the abtide may be used as a substitute for the
25 antibody in many of the reactions or assays that the antibody
could be used in. For example, the abtide could be used in
immunoassays known in the art, e.g., those designed to detect
or measure the amount of the antigen. Of course, such
immunoassays may have to be suitably modified. For example,
30 many immunoassays make use of a step in which a second
antibody, labeled with a radioactive moiety or an enzyme such
as alkaline phosphatase, specifically binds to the first
antibody. Such a second antibody would not be expected to
specifically bind to the abtide. However, it would be well
35 within the competence of one of ordinary skill in the art to
fabricate another labelling moiety, perhaps a third antibody,

that was able to specifically bind to the abtide, or to label
the abtide with a detectable marker prior to use.

The immunoassays which can be used include but are
not limited to competitive and non-competitive assay systems
5 using techniques such as western blots, radioimmunoassays,
ELISA (enzyme linked immunosorbent assays), "sandwich"
immunoassays, dot immunoblot assays, immunoprecipitation
assays, precipitin reactions, gel diffusion precipitin
reactions, immunodiffusion assays, agglutination assays,
10 complement-fixation assays, immunoradiometric assays,
fluorescent immunoassays, protein A immunoassays,
immunoaffinity chromatography, and flow dipstick assays to
name but a few. For examples of exemplary procedures which
can be used in immunoassays, see generally Kricka, 1985,
15 Clinical and Biochemical Analysis 17:1-15; Armbruster, 1993,
Clin. Chem. 39/2:181-195; Birnbaum et al., 1992, Anal.
Biochem. 206:168-171; Miyai, 1985, Adv. Clin. Chem. 24:61-110;
and references cited therein.

The samples to be assayed in the immunoassays can be
20 any sample that may contain the antigen or ligand desired to
be assayed. For example, these samples can be body fluids
such as plasma, blood, serum, saliva, cerebrospinal fluid,
synovial fluid, etc.

The detectable label to be used in the immunoassays
25 can be any detectable label known in the art. Such labels
include radioisotopes, fluorescent dyes, enzymes (for example
horseradish peroxidase or alkaline phosphatase),
chemiluminescent molecules, metal atoms, or phosphorescent
dyes, colored particles, metal and dye colloids.
30

5.5.1.1. SANDWICH ELISA

In a particular embodiment, the abtides can be used
in a sandwich enzyme immunoassay. One description of such an
embodiment, presented by way of example and not limitation,
35 follows: A molecule comprising an abtide is affixed to a
solid substratum. The molecule comprising the abtide may be
linked to a substance that will provide for greater attachment

of the molecule to the substratum. The sample to be assayed
is contacted with the molecule comprising the abtide that is
bound to the substratum. The substances in the sample that
are specific binding partners of the abtide (analyte) will
5 bind to the abtide, and non-binding sample components are
removed by washing. An enzyme-conjugated monoclonal antibody
directed against the analyte is added. This enzyme-conjugated
monoclonal antibody binds to the part of the analyte it is
specific for and completes the sandwich. After removal of
10 unbound enzyme-conjugated monoclonal antibody by washing, a
substrate solution is added to the wells. A colored product
is formed in proportion to the amount of analyte present in
the sample. The reaction may be terminated by addition of
stop solution and absorbance is measured
15 spectrophotometrically. The following illustrates these steps
in more detail.

    (a)   Polystyrene microtiter wells (Flow Laboratory)
are coated overnight at room temperature with
100 μl of a solution of a molecule comprising
an abtide at a concentration of 1 mg/ml in
20 phosphate buffered saline (PBS).

    (b)   Coating solution is discarded and wells are
blocked for 1-2 hours at room temperature with
300 μl of 1% bovine serum albumin (BSA) in
phosphate-buffered saline (PBS) with 0.05% of
25 Tween 20 (PBS-Tween buffer).

    (c)   150 μl of sample (suspected of containing an
analyte the presence or amount of which it is
desired to measure) diluted in 1% BSA-PBS is
30 added per well. Wells are incubated 1 hour at
room temperature.

    (d)   Wells are washed 4 times with PBS-Tween buffer.

    (e)   100 μl of horseradish peroxidase conjugated
monoclonal antibody specific for the analyte in
35 1% BSA-PBS is added per well. The
concentration of the monoclonal antibody can be

from about 10 ng/ml to 10 mg/ml. Wells are incubated 1 hour at room temperature.

(f) Wells are washed 6 times with PBS -Tween buffer.

5 (g) 100 μl of ABTS® Boehringer Mannheim (2,2'-Azino-di-[3-ethylbenzthiazdine sulfonate (6)] crystallized diammonium salt working solution is added per well. ABTS® stock solution is prepared at 15 mg/ml in dH$_2$O. To make the

10 working solution, 200 μl of this ABTS® stock is diluted into 10 ml of citrate phosphate buffer (17 mm citric acid, 65 mm dibasic sodium phosphate) and 10 μl 30% H$_2$O$_2$.

(h) The absorbance of each well is measured at 405

15 nm in a microtiter plate reader (Dynatech MR600, Dynatech Corp., Alexandria, VA.).

5.5.2. PHARMACEUTICAL COMPOSITIONS

The invention provides methods of treatment by

20 administration to a subject of an effective amount of a pharmaceutical (therapeutic or diagnostic) composition comprising an abtide. Such an abtide envisioned for therapeutic or diagnostic use is referred to hereinafter as a "Therapeutic" or "Therapeutic of the invention." Such

25 therapeutics are abtides that specifically bind to a molecule *in vivo*, to exert a therapeutic or diagnostic effect. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs,

30 etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed are known in the art and can be selected from among those described hereinbelow.

Various delivery systems are known and can be used

35 to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells containing the Therapeutic, receptor-

mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol.
Chem. 262:4429-4432), etc. Methods of introduction include
but are not limited to intradermal, intramuscular,
intraperitoneal, intravenous, subcutaneous, intranasal,

5 epidural, and oral routes as well as transdermal and
subcutaneous time-release implants. The Therapeutics may be
administered by any convenient route, for example by infusion
or bolus injection, by absorption through epithelial or
mucocutaneous linings (*e.g.*, oral mucosa, rectal and

10 intestinal mucosa, etc.) and may be administered together with
other biologically active agents. Administration can be
systemic or local. In addition, it may be desirable to
introduce the pharmaceutical compositions of the invention
into the central nervous system by any suitable route,

15 including intraventricular and intrathecal injection;
intraventricular injection may be facilitated by an
intraventricular catheter, for example, attached to a
reservoir, such as an Ommaya~reservoir. In a specific
embodiment, it may be desirable to utilize liposomes targeted

20 via abtides to specific identifiable cell surface antigens.

In a specific embodiment, it may be desirable to
administer the Therapeutics of the invention locally to the
area in need of treatment; this may be achieved by, for
example, and not by way of limitation, local infusion during

25 surgery, topical application, *e.g.*, in conjunction with a
wound dressing after surgery, by injection, by means of a
catheter, by means of a suppository, or by means of an
implant, said implant being of a porous, non-porous, or
gelatinous material, including membranes, such as sialastic

30 membranes, or fibers.

The present invention provides pharmaceutical
compositions. Such compositions comprise a therapeutically
effective amount of a Therapeutic, and a pharmaceutically
acceptable carrier or excipient. Such a carrier includes but

35 is not limited to saline, buffered saline, dextrose, water,
glycerol, ethanol, and combinations thereof. The carrier and

composition can be sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering

5 agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers

10 such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a

15 pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as

20 lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the

25 quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be

30 provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those

35 derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium,

calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or

5 condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the

10 route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more

15 of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the

20 agency of manufacture, use or sale for human administration.

5.5.3. IN VIVO DIAGNOSTIC AND THERAPEUTIC
USES OF ABTIDES

Another area where abtides can be used in place of

25 antibodies is in the imaging, detection, or treatment of disease. Current diagnostic and therapeutic methods make use of antibodies to target imaging agents or therapeutic substances, *e.g.* to tumors. Since abtides possess the same specificity of binding as antibodies, abtides can be used in

30 place of antibodies in such diagnostic and therapeutic methods.

Abtides may be linked to chelators such as those described in U.S. Patent No. 4,741,900 or U.S. Patent No. 5,326,856. The abtide-chelator complex may then be

35 radiolabeled to provide an imaging agent for diagnosis or treatment of disease. The abtide may also be used in the methods that are disclosed in co-pending U.S. patent

application serial no. 08/127,351 for creating a radiolabeled peptide for use in imaging or radiotherapy. This application contains a review of methods of using peptides in imaging agents.

5   In *in vivo* diagnostic applications, specific tissues or even specific cellular disorders may be imaged by administration of a sufficient amount of a labeled abtide of the instant invention.

A wide variety of metal ions suitable for *in vivo* 10 tissue imaging have been tested and utilized clinically. For imaging with radioisotopes, the following characteristics are generally desirable: (a) low radiation dose to the patient; (b) high photon yield which permits a nuclear medicine procedure to be performed in a short time period; (c) ability 15 to be produced in sufficient quantities; (d) acceptable cost; (e) simple preparation for administration; and (f) no requirement that the patient be sequestered subsequently. These characteristics generally translate into the following: (a) the radiation exposure to the most critical organ is less 20 than 5 rad; (b) a single image can be obtained within several hours after infusion; (c) the radioisotope does not decay by emission of a particle; (d) the isotope can be readily detected; and (e) the half-life is less than four days (Lamb and Kramer, "Commercial Production of Radioisotopes for 25 Nuclear Medicine", In Radiotracers For Medical Applications, Vol. 1, Rayudu (Ed.), CRC Press, Inc., Boca Raton, pp. 17-62). Preferably, the metal is technetium-99m.

By way of illustration, the targets that one may image include any solid neoplasm, certain organs such a lymph 30 nodes, parathyroids, spleen and kidney, sites of inflammation or infection (*e.g.*, macrophages at such sites), myocardial infarction or thromboses (neoantigenic determinants on fibrin or platelets), and the like evident to one of ordinary skill in the art. Furthermore, the neoplastic tissue may be present 35 in bone, internal organs, connective tissue, or skin.

As is also apparent to one of ordinary skill in the art, one may use the present invention in *in vivo* therapeutics

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(e.g., using radiotherapeutic metal complexes), especially
after having diagnosed a diseased condition via the *in vivo*
diagnostic method described above, or in *in vitro* diagnostic
application (e.g., using a radiometal or a fluorescent metal
5 complex).

Accordingly, a method of obtaining an image of an
internal region of a subject is contemplated in the instant
invention which comprises administering to a subject an
effective amount of an abtide composition containing a metal
10 in which the metal is radioactive, and recording the
scintigraphic image obtained from the decay of the radioactive
metal. Likewise, a method is contemplated of enhancing an MR
image of an internal region of a subject which comprises
administering to a subject an effective amount of an abtide
15 composition containing a metal in which the metal is
paramagnetic, and recording the MR image of an internal region
of the subject.

Other methods include a method of enhancing a
sonographic image of an internal region of a subject
20 comprising administering to a subject an effective amount of
an abtide composition containing a metal and recording the
sonographic image of an internal region of the subject. In
this latter application, the metal is preferably any non-toxic
heavy metal ion. A method of enhancing an X-ray image of an
25 internal region of a subject is also provided which comprises
administering to a subject an abtide composition containing a
metal, and recording the X-ray image of an internal region of
the subject. A radioactive, non-toxic heavy metal ion is
preferred.

30 The use of abtides in place of antibodies in such
methods has certain advantages. Because abtides are peptides
rather than large proteins such as antibodies, the kinetics of
their distribution in the body and clearance from the
bloodstream differ from that of large proteins such as
35 antibodies. For example, as demonstrated in Section 6.1.4,
abtides can be used for *in vivo* imaging of disease states in

about 2 to 5 hours. Current methods of tumor imaging using antibodies require approximately 24 to 48 hours.

Because abtides are peptides, they are cleared from the blood faster than antibodies. This means that there will
5 be less background signal in the bloodstream when using abtides to image disease states than there is when using antibodies.

Peptides most likely will provoke less of an immune response in patients than do large proteins such as
10 antibodies. This consideration is especially important when diagnosis or treatment is required to be done repeatedly or over a long period of time.

Abtides, because they are generally small proteins, can remain soluble in physiological fluids under conditions
15 where antibodies cannot.

Abtides, again because they are generally peptides, can be produced synthetically or by recombinant methods and therefore may be less costly to produce than antibodies.

Abtides may be used individually. Alternatively,
20 abtides may be used as compositions of abtides in which the peptide sequences of the abtides differ.

5.6. SYNTHESIS OF PEPTIDES

25   5.6.1. PROCEDURE FOR SOLID PHASE SYNTHESIS
Abtide or mimetope peptides may be prepared by methods that are known in the art. For example, in brief, solid phase peptide synthesis consists of coupling the carboxyl group of the C-terminal amino acid to a resin and
30 successively adding N-alpha protected amino acids. The protecting groups may be any known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. The coupling of amino acids to appropriate resins is
35 described by Rivier et al., U.S. Patent No. 4,244,946. Such solid phase syntheses have been described, for example, by Merrifield, 1964, J. Am. Chem. Soc. 85:2149; Vale et al.,

1981, Science 213:1394-1397; Marki et al., 1981, J. Am. Chem. Soc. 103:3178 and in U.S. Patent Nos. 4,305,872 and 4,316,891. In a preferred aspect, an automated peptide synthesizer is employed.

5   By way of example but not limitation, peptides can be synthesized on an Applied Biosystems Inc. ("ABI") model 431A automated peptide synthesizer using the "Fastmoc" synthesis protocol supplied by ABI, which uses 2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium

10 hexafluorophosphate ("HBTU") (R. Knorr et al., 1989, Tet. Lett., 30:1927) as coupling agent. Syntheses can be carried out on 0.25 mmol of commercially available 4-(2',4'-dimethoxyphenyl-(9-fluorenyl-methoxycarbonyl)-aminomethyl)-phenoxy polystyrene resin ("Rink

15 resin" from Advanced ChemTech) (H. Rink, 1987, Tet. Lett. 28:3787). Fmoc amino acids (1 mmol) are coupled according to the Fastmoc protocol. The following side chain protected Fmoc amino acid derivatives are used: FmocArg(Pmc)OH; FmocAsn(Mbh)OH; FmocAsp(^{t}Bu)OH; FmocCys(Acm)OH;

20 FmocGlu(^{t}Bu)OH; FmocGln(Mbh)OH; FmocHis(Tr)OH; FmocLys(Boc)OH; FmocSer(^{t}Bu)OH; FmocThr(^{t}Bu)OH; FmocTyr(^{t}Bu)OH. [Abbreviations: Acm, acetamidomethyl; Boc, tert-butoxycarbonyl; ^{t}Bu, tert-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; Mbh, 4,4'-dimethoxybenzhydryl;

25 Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Tr, trityl].
          Synthesis is carried out using N-methylpyrrolidone (NMP) as solvent, with HBTU dissolved in N,N-dimethylformamide (DMF). Deprotection of the Fmoc group is effected using ca. 20% piperidine in NMP. At the end of each synthesis the

30 amount of peptide present is assayed by ultraviolet spectroscopy. A sample of dry peptide resin (ca. 3-10 mg) is weighed, then 20% piperidine in DMA (10 mL) is added. After 30 min sonication, the UV (ultraviolet) absorbance of the dibenzofulvene-piperidine adduct (formed by cleavage of the

35 N-terminal Fmoc group) is recorded at 301 nm. Peptide substitution (in mmol g^{-1}) can be calculated according to the equation:

$$\text{substitution} = \frac{A \times v}{7800 \times w} \times 1000$$

where A is the absorbance at 301 nm, v is the volume of 20%
piperidine in DMA (in mL), 7800 is the extinction coefficient
(in $mol^{-1}dm^3cm^{-1}$) of the dibenzofulvene-piperidine adduct, and w
is the weight of the peptide-resin sample (in mg).

Finally, the N-terminal Fmoc group is cleaved using
20% piperidine in DMA, then acetylated using acetic anhydride
and pyridine in DMA. The peptide resin is thoroughly washed
with DMA, CH_2Cl_2 and finally diethyl ether.

5.6.2. CLEAVAGE AND DEPROTECTION

By way of example but not limitation, cleavage and
deprotection can be carried out as follows: The air-dried
peptide resin is treated with ethylmethyl-sulfide (EtSMe),
ethanedithiol (EDT), and thioanisole (PhSMe) for approximately
20 min. prior to addition of ~95% aqueous trifluoracetic acid
(TFA). A total volume of ca. 50 mL of these reagents are used
per gram of peptide-resin. The following ratio is used: TFA
: EtSMe : EDT : PhSme (10 : 0.5 : 0.5 : 0.5). The mixture is
stirred for 3 h at room temperature under an atmosphere of N_2.
The mixture is filtered and the resin washed with TFA (2 x 3
mL). The combined filtrate is evaporated in vacuo, and
anhydrous diethyl ether added to the yellow/orange residue.
The resulting white precipitate is isolated by filtration.
See King et al., 1990, Int. J. Peptide Protein Res. 36:255-266
regarding various cleavage methods.

5.6.3. PURIFICATION OF THE PEPTIDES

Purification of the synthesized peptides can be
carried out by standard methods including chromatography
(e.g., ion exchange, affinity, and sizing column
chromatography, high performance liquid chromatography
(HPLC)), centrifugation, differential solubility, or by any
other standard technique.

5.6.4. CONJUGATION OF PEPTIDES TO OTHER MOLECULES

The abtides of the present invention may be linked to other molecules (e.g., a detectable label, a molecule facilitating adsorption to a solid substratum, or a toxin,
5 according to various embodiments of the invention) by methods that are well known in the art. Such methods include the use of homobifunctional and heterobifunctional cross-linking molecules.

The homobifunctional molecules have at least two
10 reactive functional groups, which are the same. The reactive functional groups on a homobifunctional molecule include, for example, aldehyde groups and active ester groups. Homobifunctional molecules having aldehyde groups include, for example, glutaraldehyde and subaraldehyde. The use of
15 glutaraldehyde as a cross-linking agent was disclosed by Poznansky et al., 1984, Science 223:1304-1306.

Homobifunctional molecules having at least two active ester units include esters of dicarboxylic acids and N-hydroxysuccinimide. Some examples of such N-succinimidyl
20 esters include disuccinimidyl suberate and dithio-bis-(succinimidyl propionate), and their soluble bis-sulfonic acid and bis-sulfonate salts such as their sodium and potassium salts. These homobifunctional reagents are available from Pierce, Rockford, Illinois.

25 The heterobifunctional molecules have at least two different reactive groups. Some examples of heterobifunctional reagents containing reactive disulfide bonds include N-succinimidyl 3-(2-pyridyl-dithio)propionate (Carlsson et al., 1978, Biochem J. 173:723-737), sodium S-4-
30 succinimidyloxycarbonyl-alpha-methylbenzylthiosulfate, and 4-succinimidyloxycarbonyl-alpha-methyl-(2-pyridyldithio)toluene. N-succinimidyl 3-(2-pyridyldithio)propionate is preferred. Some examples of heterobifunctional reagents comprising reactive groups having
35 a double bond that reacts with a thiol group include succinimidyl 4-(N-maleimidomethyl)cyclohexahe-1-carboxylate and succinimidyl m-maleimidobenzoate.

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Other heterobifunctional molecules include
succinimidyl 3-(maleimido)propionate, sulfosuccinimidyl 4-(p-maleimido-phenyl)butyrate, sulfosuccinimidyl 4-(N-maleimidomethyl-cyclohexane)-1-carboxylate, maleimidobenzoyl-N-hydroxy-succinimide ester. The sodium sulfonate salt of succinimidyl m-maleimidobenzoate is preferred. Many of the above-mentioned heterobifunctional reagents and their sulfonate salts are available from Pierce.

Additional information regarding how to make and use these as well as other polyfunctional reagents may be obtained from the following publications or others available in the art:

Carlsson et al., 1978, Biochem. J. 173:723-737.

Cumber et al., 1985, Methods in Enzymology 112:207-224.

Jue et al., 1978, Biochem 17:5399-5405.

Sun et al., 1974, Biochem. 13:2334-2340.

Blattler et al., 1985, Biochem. 24:1517-152.

Liu et al., 1979, Biochem. 18:690-697.

Youle and Neville, 1980, Proc. Natl. Acad. Sci. USA 77:5483-5486.

Lerner et al., 1981, Proc. Natl. Acad. Sci. USA 78:3403-3407.

Jung and Moroi, 1983, Biochem. Biophys. Acta 761:162.

Caulfield et al., 1984, Biochem. 81:7772-7776.

Staros, 1982, Biochem. 21:3950-3955.

Yoshitake et al., 1979, Eur. J. Biochem. 101:395-399.

Yoshitake et al., 1982, J. Biochem. 92:1413-1424.

Pilch and Czech, 1979, J. Biol. Chem. 254:3375-3381.

Novick et al., 1987, J. Biol. Chem. 262:8483-8487.

Lomant and Fairbanks, 1976, J. Mol. Biol. 104:243-261.

Hamada and Tsuruo, 1987, Anal. Biochem. 160:483-488.

Hashida et al., 1984, J. Applied Biochem. 6:56-63.

Additionally, methods of cross-linking are reviewed by Means and Feeney, 1990, Bioconjugate Chem. 1:2-12.

5.6.4.1. BIOTINYLATION OF PEPTIDES

Methods of biotinylating peptides are well known in the art. Any convenient method may be employed in the

practice of the invention. For example, the following
procedure was used:

> (1)   dissolve 10 mg of peptide in 100 μL of 0.1 %
> acetic acid;
>
> (2)   add 900 μL of PBS;
>
> (3)   add 3.3 mg of biotin-LC-NHS (Pierce, Rockford,
> IL);
>
> (4)   incubate for 30 minutes at room temperature;
>
> (5)   purify over a Superose 12 column (Pharmacia,
> Piscataway, NJ).

6.   EXAMPLES

6.1.   ABTIDES MIMICKING THE BINDING SPECIFICITY OF MONOCLONAL ANTIBODY 7E11-C5

6.1.1.   IDENTIFICATION AND ISOLATION OF ABTIDES MIMICKING THE BINDING SPECIFICITY OF MONOCLONAL ANTIBODY 7E11-C5

7E11-C5 is a murine IgG1 monoclonal antibody
specific for an antigen of a human prostate carcinoma, LNCaP.
7E11-C5 binds strongly to malignant prostatic epithelium but
only weakly to normal prostatic epithelium. It does not bind
to non-prostatic tumors or to most normal organs. See
Horoszewicz et al., 1987, Anticancer Res. 7:927-936. 7E11-C5
is also described in U.S. Patent No. 5,162,504 issued November
10, 1992. Hybridomas producing monoclonal antibody 7E11-C5
were grown as ascites in mice and 7E11-C5 was purified from
ascites fluid by Protein A affinity chromatography to over 90%
purity as judged by sodium dodecylsulfate polyacrylamide gel
electrophoresis.

In order to identify abtides mimicking binding
specificity of monoclonal antibody 7E11-C5, monoclonal
antibody 7E11-C5 was used as the target ligand in a first
screening of the TSAR-9 library (see Kay et al., 1993, Gene
128:59-65 and PCT publication WO 94/18318, dated August 18,
1994). The following screening procedure was used. First,

7E11-C5 was bound to a well of a microtiter plate. 7E11-C5 at
a concentration of 11.2 mg/mL in phosphate buffered saline
(PBS), pH 6.0, was diluted to 100 μg per mL in 0.1x PBS pH
7.2. One hundred microliters (100 μL) of this dilution was
5 added to one well of a microtiter plate, and allowed to
incubate for 1-6 hours at room temperature or overnight at
4° C. After incubation, the well was washed at least 4
times with a blocking buffer which consisted of either 1%
bovine serum albumin (BSA) in PBS, 1% non-fat dry milk
10 (NFDM) in PBS, or 0.1% Tween® in either 1% BSA in PBS or 1%
NFDM in PBS. Two hundred microliters of the blocking
buffer was then added to the well and allowed to incubate
for at least an hour at room temperature.

Next, an aliquot of the TSAR-9 library was added
15 to the well containing bound 7E11-C5. An aliquot of the
library containing 10^{10} phage particles was added to the
well and allowed to incubate for at least 1 hour at room
temperature. This resulted in the binding to the plate of
those phage containing binding domains that bind to 7E11-
20 C5. After an hour, the well was washed extensively with
either 1% bovine serum albumin (BSA) in PBS, 1% non-fat dry
milk (NFDM) in PBS, or 0.1% Tween® in either 1% BSA in PBS
or 1% NFDM in PBS.

25 After washing, phage bound to the 7E11-C5
antibody in the well were eluted by adding 100 μL of an
acid solution of 0.2 M glycine-HCl, pH 2.0. After
incubation from 15 minutes to 1 hour, the acid solution
containing eluted phage was transferred to a 1.5 mL
30 microfuge tube, and an equal volume of 0.2 M Tris-HCl, pH
7.5 added to neutralize the acid solution. In some cases,
the neutralized phage solution was immediately added to a
second well containing bound 7E11-C5 antibody, and the
binding and elution procedure repeated.

35 If it was desired that the level of enrichment be
monitored during the above steps, an irrelevant phage that

does not bind 7E11-C5 but that expresses the β-galactosidase gene was added to the aliquot from the TSAR-9 library. This phage gives rise to blue plaques when plated in the presence of X-Gal and IPTG. Following a screening
5 step, the eluted phage were plated in X-gal and IPTG. An aliquot of unscreened phage were plated as well. The ratio of white to blue plaques was measured for both phage samples. The increase in the proportion of white plaques (from the TSAR-9 phage that bind to 7E11-C5) to blue
10 plaques (from the irrelevant phage) indicated the degree to which the screening process enriched the population of phage for those phage that bind 7E11-C5.

If it was desired that the specificity of binding be monitored during the above screening steps, screening
15 was done against an irrelevant target (either BSA, mouse IgG, or plastic) in addition to being done against 7E11-C5. The enrichment of white plaques over blue plaques when panning was done against 7E11-C5 rather than an irrelevant
20 target indicated the level of specificity of binding.

After screening, the phage were amplified by adding an aliquot of the eluted phage to a solution containing LB broth and competent DH5αF' E. coli cells (GIBCO BRL, Gaithersburg, MD). Typically a
25 2-5 μL aliquot of the phage solution was added to 125 μL of LB broth containing a 1:50 dilution of DH5αF' E.Coli cells (about 1 x 10^9 cells/ml). 3.3 mL of top agar was added to this solution, and the mixture was plated out onto a Petri dish containing agar. Often the phage were titered by
30 making several serial 1:10 dilutions, and plating out the dilution as described above. After incubation overnight at 37° C, the plates were evaluated for growth of plaques, and counted if desired. The plates were eluted by adding 3-5 mL of 100 mM NaCl, 10 mM MgCl$_2$, 10 mM Tris-HCl, pH 7.5 (SM
35 buffer) to each plate and incubating for 1-5 hours with gentle rocking. The solution was then removed from the

plate, centrifuged, and either stored, amplified further, or analyzed.

In some cases, the phage were amplified in solution by adding 1-5 µL of the phage solution to 1-5 mL
5 of LB broth containing a 1:50 or 1:100 dilution of competent DH5αF' *E. coli* cells (about 1 x 10⁹ cells/ml. After incubation for either 6 hours or overnight, the solution was centrifuged and the supernatant collected. In some cases, the phage particles were
10 precipitated with polyethylene glycol (PEG) by adding a 1/5 volume of PEG to the clarified phage solution, and incubating for 1 hour on ice. After centrifugation, the phage were usually reconstituted with 100 µL of SM buffer.

Using the above procedures, nine different phage
15 were isolated that expressed peptides containing binding domains that were capable of binding monoclonal antibody 7E11-C5. Molecules comprising these binding domains are thus mimetopes of the antigen recognized by the monoclonal
20 antibody 7E11-C5. The binding domains of the peptides expressed by the nine phage were sequenced according to standard methods of DNA sequencing (Sequenase ™, U.S. Biochemical Corp., Cleveland, OH). The determination of those DNA sequences allowed the determination of the amino
25 acid sequences of these mimetopes. These sequences are shown in Table 1. Examination of these amino acid sequences showed that they shared a common motif of MYxxLH (SEQ ID NO. 10).

30

35

TABLE 1

SCVSHMLDTSRVYTAYANPG	MYSRLH	SPAVRPLTQSSA	(SEQ ID No.: 11)
SVQFKSISSRSMDDVVKDPGPKPA	MYNRLH	SKNPFTLS	(SEQ ID No.: 12)
YFDHTYSGPVVKNGGLVSPGVLS	MYNRLH	SDGGPSLAS	(SEQ ID No.: 13)
TVAT	MHDRLH	SAPGSGNLPGSYDIKPIFKAQSGALHST	(SEQ ID No.: 14)
IDMPQTAST	MYNMLH	RNEPGGRKLSPPANDMPPALLKR	(SEQ ID No.: 15)
RLGNHVWREGGG	MYQQLH	HNFP	(SEQ ID No.: 16)
RDSAVENPSVGGEIP	MYRYLH	QR	(SEQ ID No.: 17)
PVQKEYGFGMSGAS	MIRLLR	ETP	(SEQ ID No.: 18)
QKGGPGLLLYGGDS	MYITLH	EPG	(SEQ ID No.: 19)
	MYxxLH		(SEQ ID No.: 10)

LYANPGMYSRLHSPA   (SEQ ID No: 20)

In order to use the mimetopes to identify abtides, peptides corresponding to the mimetope sequences were synthesized, and then dissolved in either water or PBS to give a final concentration ·of 5 μg/mL. Specifically, a peptide

5 called 7E11-9.5, with the sequence LYANPGMYSRLHSPA (SEQ ID NO: 20) and a peptide with the sequence GMYSRLHSPA (SEQ ID NO: 21) were synthesized.

First the mimetope peptide 7E11-9.5 was tested for its ability to bind to the monoclonal antibody 7E11-C5.

10 Ninety-six well plates (Immunlon 4, Dynatech, Alexandria, VA) were coated with 50 μL of a 5 μg/mL solution of the mimetope peptide 7E11-9.5 and incubated overnight at room temperature. Following incubation, the wells were washed 4 times with 1% BSA in PBS. Biotinylated monoclonal antibody

15 7E11-C5 was serially diluted with PBS beginning with a concentration of 29 μg/mL and various amounts of the monoclonal antibody were added to the wells that had been coated with the mimetope peptide. After incubating for 1 hour at room temperature, the wells were washed four times

20 with 1% BSA in PBS and 2 times with PBS. Then, 100 μL of a 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was added to each well. After an hour, the plate was again washed 4 times

25 with PBS and 100 μL of a 1 mg/mL solution of the enzyme substrate p-nitrophenyl phosphate was added to each well. Color was allowed to develop for 15 minutes to 1 hour and absorbance was read at 405 nm. Figure 2 shows the results. It can be seen from Figure 2 that monoclonal antibody 7E11-

30 C5 binds to the synthesized mimetope 7E11-9.5 in a concentration dependent manner.

Next, the mimetope peptide was used to isolate abtides from a peptide library. Fifty to one hundred microliters of the solution of this peptide was used to

35 coat the wells of a 96-well plate, the wells were blocked with 0.5 % BSA in PBS, and the wells were used for

screening. An aliquot of the TSAR-9 random phage library
(containing approximately 3 x 10^{10} phage particles) was used
as in the initial screening, and 4 rounds of screening were
performed. After the first two rounds, the phage were
5 amplified. Two more rounds of screening were then
performed. By this procedure, phage from the TSAR-9
library that expressed peptides capable of binding to the
7E11-9.5 mimetope peptide were identified and isolated.
The peptides containing the binding domains of these phage
10 are abtides and were discovered to mimic the binding
specificities of monoclonal antibody 7E11-C5. These
abtides are termed "7E11-C5 abtides."

Phage encoding the 7E11-C5 abtides were subjected
to DNA sequencing of the nucleotide sequences encoding
15 their binding peptides in order to obtain the DNA and amino
acid sequences of the 7E11-C5 abtides. Table 2 shows the
amino acid sequence of five-of the 7E11-C5 abtides that had
relatively high affinity for the mimetope.

20

TABLE 2

Clone	Sequence
14	GIINANDPLPFWFMSPYTPGPAPIDINASRALVSNESG
17	DLSRNLDFGRFLLYNAYVPGFTPTFISLTAEHLSSPKG
15	CGRAYCLSGNYNIFGALFPGVSTPYADVGHDDAQSWRR
13	RCSPIWGISYPFGLLSSNPGVCHSSDAETNIRNDILTT
16	GHSNYCFVSTLGMPIVGFPSINARGLIHYGGSDPRLAA

35 The amino acid sequence shown in Table 2 for clone
14 is SEQ ID NO: 1. The amino acid sequence shown in Table 2
for clone 17 is SEQ ID NO: 2. The amino acid sequence shown

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in Table 2 for clone 15 is SEQ ID NO: 3. The amino acid
sequence shown in Table 2 for clone 13 is SEQ ID NO: 4. The
amino acid sequence shown in Table 2 for clone 16 is SEQ ID
NO: 5.

5   It was of interest to determine whether there might
be some structural basis for the similarity in binding
characteristics between the monoclonal antibody 7E11-C5 and
the 7E11-C5 abtides. The amino acid sequences of the
complementarity determining regions (CDRs) of the monoclonal
10 antibody 7E11-C5 were determined by sequencing cDNA clones of
the genes encoding the variable regions of the antibody.
These CDRs are responsible for the specific binding of
monoclonal antibody 7E11-C5 to its antigen. Figure 3 presents
a comparison of the amino acid sequences of the abtides of
15 Table 2 and portions of the amino acid sequences of some of
the CDRs of monoclonal antibody 7E11-C5. Surprisingly, it can
be seen that there are similarities in the sequences of these
abtides and the sequences of the CDRs of the monoclonal
antibody.
20
        6.1.2.   CHARACTERIZATION OF 7E11-C5 ABTIDES
            7E11-C5 abtides were tested for their ability to
bind to the 7E11-C5 mimetopes that were used as target ligands
in the second screening step above. The DNA sequences of the
25 regions of the phage DNA encoding the abtides were determined.
This allowed the determination of the amino acid sequences of
the abtides. Based upon these determined amino acid
sequences, synthetic peptides corresponding to these sequences
were made. These synthetic peptides (7E11-C5 abtides) were 38
30 amino acids in length.

        6.1.2.1.   DOT BLOTS USING 7E11-C5 ABTIDES
            In some cases, these abtides were used in a dot blot
experiment. In those cases, 1 μL of a 1 mg/mL solution of the
35 38-residue abtides was spotted onto nitrocellulose (0.2 μm or
0.45 μm, Schleicher & Schuell, Keene, NH) strips or
circles. After drying (about 1/2 hour), the nitrocellulose

was blocked for 1 hour in a solution of 1% BSA in PBS. The nitrocellulose was then allowed to incubate in approximately 5 mL of a solution of 0.1 mg/mL of a biotinylated 7E11-9.5 mimetope peptide (biotin-

5 LYANPGMYSRLHSPA). This mimetope peptide was one of those described in Section 6.1.1 above that were synthesized based upon the nine peptides that were identified in the screening of Section 6.1.1 above. After an hour, the nitrocellulose was washed approximately 5 times with a

10 solution of 1% BSA in PBS. A 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was then added and allowed to incubate for 1 hour, after which the nitrocellulose was again washed extensively. Finally, a solution of 5-bromo-

15 4-chloro-3-indolyl phosphate (0.15 mg/mL) and nitro blue tetrazolium (0.3 mg/mL) (Sigma, St Louis, MO) (BCIP/NBT) was added as an enzyme substrate. Color was allowed to develop and the absorbance at 405 nm was read.

20   An example of such a dot blot assay is shown in Figure 4. In Figure 4, the 7E11-C5 abtides known as clone 14, clone 17, clone 15, clone 16, and clone 13 were tested for their ability to bind the biotinylated 7E11-9.5 mimetope peptide. Also tested, as a positive control, was

25 the monoclonal antibody 7E11-C5. 7E11-C5 was spotted onto the region marked 351 in Figure 4. Inspection of Figure 4 shows that at least three of the abtides (clone 14, clone 17, and clone 15) bound the mimetope. This shows that these abtides are capable of mimicking the specific binding

30 exhibited by the monoclonal antibody 7E11-C5.

        6.1.2.2. 7E11-C5 ABTIDES IN PLACE OF
            ANTIBODIES IN IMMUNOASSAYS

        The ability of abtides synthesized having the

35 amino acid sequence encoded by the random inserts of the

phage that bound the 7E11-9.5 mimetope was further
evaluated by ELISA assay methods.

The 7E11-C5 abtides clone 14 and clone 17 (See
Table 2) were each dissolved in 0.1X PBS to give a solution
5 of 5 μg/mL. Fifty microliters of each of these solutions
was used to coat the wells of a 96-well microtiter plate
(Immulon 4, Dynatech, Alexandria, VA) by overnight
incubation at 4° C. Following this incubation, the abtide
solutions were removed and the wells were blocked with 200
10 μL μl of a solution of 1% BSA in PBS. Mimetope peptides
7E11-9.5 (LYANPGMYSRLHSPA [SEQ IN NO: 20]) and GMYSRLHSPA
(SEQ ID NO: 21) were biotinylated as described in Section
5.6.4.1 and dissolved in H$_2$O to give 1 mg/mL solutions.
Serial 1:2 dilutions were made of these solutions and these
15 dilutions were added to the wells of the microtiter plate
containing the bound abtides. After incubation for 1 hour
at room temperature, the wells were washed four times with
1% BSA in PBS. Then a 1:2000 dilution of Extravidin-
20 Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis,
MO) in PBS was added to each well and incubated for 1 hour
at room temperature. Following incubation, the wells were
washed four times with 1% BSA in PBS and then twice in PBS.
One hundred microliters of a 1 mg/mL solution of p-
25 nitrophenyl phosphate in diethanol amine (DEA) buffer (both
from Kirkegaard & Perry Laboratories, Gaithersburg, MD) was
then added and, after incubation for 15-30 minutes at room
temperature, the absorbance of the solutions in the wells
was read at 405 nm. The results are shown in Figure 5.
30 Figure 5 shows that, except for the non-linear
effect at high concentrations of mimetope, there is a good
correlation between the amount of mimetope added to the
wells and the absorbance at 405 nm. The use of antibodies
in assays such as enzyme-linked immunosorbent assays
35 (ELISAs) to measure the concentration of a substance is
well known in the art. The ability of antibodies to

specifically bind their antigens is crucial to the success of such assays. Since abtides also specifically bind to antigens, it was of interest to determine if abtides can be used in place of antibodies in immunoassays such as ELISA-like assays to measure the concentration of a substance. The results of Figure 5 show that the abtides of the present invention can be used in assays in much the same way that antibodies can be used in immunoassays such as ELISAs.

6.1.2.3. BIOTINYLATION OF ANTIBODIES

Methods of biotinylating antibodies are well known in the art. Any convenient method may be employed in the practice of the invention. For example, the following procedure was used:

(1) dissolve 10 mg of antibody in 1 mL of PBS;

(2) add 0.44 mg of biotin-LC-NHS (Pierce, Rockford, IL);

(3) incubate for 30 minutes at room temperature;

(4) purify over a Superose 12 column (Pharmacia, Piscataway, NJ).

6.1.3. 7E11-C5 ABTIDES AND MONOCLONAL ANTIBODY 7E11-C5 RECOGNIZE AN LNCaP ANTIGEN

The monoclonal antibody 7E11-C5 recognizes a prostate specific mucin antigen of the human prostate cancer cell line LNCaP (Horoszewicz et al., 1987, Anticancer Res. 7:927-936). To determine if 7E11-C5 abtides recognize and bind the native antigen, the following experiment was done.

A sandwich assay using the LNCaP antigen was performed. The wells of a microtiter plate were coated with either monoclonal antibody 7E11-C5, the clone 14 7E11-C5 abtide, or, as a negative control, BSA. Coating and washing was as for the assay described in Section 6.1.2.2.

One hundred microliters of a lysate of LNCaP cells was added to the wells. The LNCaP lysate was prepared as described in PCT publication WO 94/18318, dated August 18, 1994. Following capture of the lysate on the plate, 100 µL

5 of a 5µg/mL solution of biotinylated 7E11-C5 monoclonal antibody was added to each well. Following incubation and washing as in Section 6.1.2.2, a 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was added to each well and incubated

10 for 1 hour at room temperature. Following incubation, the wells were washed four times with 1% BSA in PBS and then twice in PBS. One hundred microliters of a 1 mg/mL solution of p-nitrophenyl phosphate in diethanol amine (DEA) buffer (both from Kirkegaard & Perry Laboratories,

15 Gaithersburg, MD) was then added and, after incubation at room temperature for 15-30 minutes, the absorbance of the solutions in the wells was read at 405 nm.

The results are shown in Figure 6. Figure 6

20 shows that the 7E11-C5 abtide is capable of recognizing the native 7E11-C5 antigen in LNCaP lysates. This was a surprising discovery and would not have been predicted by those skilled in the art. It was generally felt that screening a library with a mimetope could yield a binder to

25 that mimetope. Whether such a binder could also bind the native epitope that the mimetope mimics was unknown. The mimetope could have represented the epitope in a loose fashion, e.g. its primary sequence could be slightly modified; its secondary structure or factors influencing

30 the presentation of the mimetope could be different from those presenting the native epitope. In such a case, the binder to the mimetope would not be specific for the native epitope. The foregoing is presented as possible explanation and not as a limitation of the present

35 invention.

6.1.4. USE OF 7E11-C5 ABTIDES IN BIODISTRIBUTION STUDIES

The 7E11 abtides described in Section 6.1 and its subsections above were used in biodistribution studies to assess their ability to target human prostate carcinoma LNCaP xenograft tumors that had been transplanted into mice.

Male SCID mice (C.B-17/Icr Tac - SCID mice) were purchased from Fox Chase (Philadelphia, PA) or Taconic Farms (Germantown, NY), were housed in sterilized cages with filter bonnets, and were given autoclaved laboratory rodent chow (Purina, St. Louis, MO), and filtered tap water *ad libitum*.

1×10^7 cells of the human prostate tumor line LNCaP (Horoszewicz et al., 1987, Anticancer Res. 7:927-936) were injected subcutaneously (s.c.) into the left rear flank of the mice. The cells were growing in exponential phase before harvesting and had been resuspended in 0.2 mL of sterile saline. Tumors were grown in the mice for 2-3 months before abtides were injected into the mice.

For biodistribution studies, abtides were modified at their amino termini with the chelator diethylene-triamine-pentaacetic acid anhydride (DTPA-A) (Sigma, St. Louis, MO). Approximately 2 mg of each abtide was initially dissolved in an appropriate volume of 0.1% acetic acid and then 1 mL of 0.1 M sodium bicarbonate, pH 8.0, was added. Two mg of DTPA-A was suspended in 100 μL of dimethylsulfoxide (DMSO), and 10 μL of the abtide solution added to this DTPA-A suspension. After 5 min incubation at room temperature, the suspension was filtered through a 0.2 μm polyvinylidene difluoride (PVDF) sample filter (Acrodisc, Gelman Sciences, Inc., Ann Arbor, MI), and purified using a Superose-12 FPLC column (Pharmacia, Piscataway, NJ) with PBS as the running buffer. Modified peptides were stored frozen at -20° C or -70° C.

Abtides modified with DTPA were labeled with $^{111}InCl_2$ as follows. 0.1 to 0.5 mCi of $^{111}InCl_2$ (Amersham, Chicago, IL) were first neutralized by adding an equal volume of 0.1 M NaOAc, and then added to 100 to 200 μg of
5 the DTPA-A-modified abtide. After incubation for one half hour, the labeled peptide was purified using a Superose-12 FPLC column (Pharmacia, Piscataway, NJ) with PBS as the running buffer. Labeled fractions were collected in a fraction collector. Tubes containing the labeled peptide
10 were pooled and used to prepare syringes for injection into mice.

In one experiment, abtide clone 14-DPTA-^{111}In (see Table 2) was injected intravenously (i.v.) into two groups of mice bearing measurable LNCaP xenografts. About 0.2 mL
15 of a 10 μg/mL solution of the radioactively labeled abtide in sterile saline was used. The specific activity of the abtide was about 32 μCi/μg. Thus, the total injected dose of radioactivity was about 120-140 x 10^6 cpm.

20 The first group of mice was sacrificed 2 hours after injection of the abtide and tissues were dissected for analysis. The second group was sacrificed 4 hours after injection. Dissected tissues were weighed and the amount of ^{111}In in them was determined by gamma counting.
25 The cpm per gram of each tissue was calculated by dividing the cpm of ^{111}In found in the tissue by the weight in grams of the tissue. The data are presented as the ratio of the cpm/g in each organ to the cpm/g in blood (organ to blood ratio). This gave the ratios that are shown in Table 3 and
30 Figure 7.

35

TABLE 3

BIODISTRIBUTION OF ABTIDE CLONE 14-DPTA-^{111}In IN LNCaP XENOGRAFT BEARING MICE

Tissue[c]	Group 1[a]		Group 2[b]	
	AVG	s.e.m.	AVG	s.e.m.
Blood	1.00	0.00	1.00	0.00
Lung	0.81	0.04	1.06	0.26
Spleen	0.83	0.16	1.42	0.74
Liver	0.95	0.04	2.12	0.69
Kidney-R	55.85	10.22	171.73	77.97
Kidney-L	53.03	11.97	182.79	86.38
Tumor	1.85	0.80	3.91	2.85
Muscle	0.33	0.03	0.42	0.01
Testes-R	0.54	0.02	0.94	0.25
Testes-L	0.68	0.24	0.88	0.24

[a] Group 1: sacrificed at 2 hours; n=2.
[b] Group 2: sacrificed at 4 hours; n=2.
[c] Value shown is the organ to blood ratio.

Table 3 and Figure 7 show that, with the exception of kidney, the highest organ to blood ratio is found in the tumor, both at 2 hours and at 4 hours post-injection of abtide. This result shows that abtides with the binding specificity of antibodies, e.g. that are specific for tumor antigens, can be used to localize to those tumors.

No unusual localization was seen to any non-tumor tissue or organ except kidney. The ratio for kidney is extremely high due to the well known tendency of injected peptides to localize to the kidneys prior to being cleared from the body.

In another experiment, abtide clone 17-DPTA-[111]In (see Table 2) was injected intravenously into four SCID mice bearing measurable LNCaP xenografts. Administration of xenografts was as above. About 0.2 mL of a 0.1 μg/mL solution
5 of the radioactively labeled clone 17 abtide in sterile saline was injected. The specific activity of the abtide was about 2.4 μCi/ng. Thus, the total injected dose of radioactivity was about $100-110 \times 10^6$ cpm.

In this experiment, mice were sacrificed at either 2
10 or 5 hours post-injection with labeled abtide. Again, as above, the data are presented as organ to blood ratios. As shown in Table 4 and Figure 8, abtide clone 17-DPTA-^{111}In localized to LNCaP xenograft tumors in mice.

15 TABLE 4

BIODISTRIBUTION OF ABTIDE CLONE
17-DPTA-^{111}In IN LNCαP-XENOGRAFT BEARING MICE

TISSUE[a]	GROUP 1 MOUSE #		GROUP 2 MOUSE #	
	1	6	2	3
BLOOD	1.00	1.00	1.00	1.00
LUNG	2.52	2.57	4.33	2.47
SPLEEN	5.82	3.00	5.53	3.70
LIVER-S	5.42	5.58	8.37	4.03
KIDNEY-R	235.63	234.88	321.09	106.74
KIDNEY-L	563.71	220.69	424.64	104.09
TUMOR-S	10.60	15.01	8.36	2.90
MUSCLE	0.93	2.96	1.16	3.04
TESTES-R	2.71	2.19	2.31	3.15
TESTES-L	1.64	1.14	5.36	3.41

[a] Value shown is the organ to blood ratio.

Table 4 and Figure 8, like Table 3 and Figure 7, show that the injected abtide localized to the tumor. This indicates that abtides can be useful in the localization of tumors.

In contrast to the results of the two experiments described above, in which radioactively labeled abtides were shown to localize to tumors, when the same experiments were done with a radioactively labeled control (non-abtide) peptide (the tripeptide GYK-DPTA), no specific localization to tumors was observed. This can be seen in Figure 9, which shows the biodistribution results for experiments using the ^{111}In-labeled control peptide.

The radiolabeled peptide conjugate GYK-DPTA-^{111}In was injectd intravenously into 5 SCID mice bearing measurable LNCaP xenografts. Mice were dissected 2 hours (n=2) and 5 hours (n=3) after injection of 1.5 μg of control peptide having a specific activity of 30 μCi/μg. The organ to blood ratios are presented in Table 5 and Figure 9. As shown, the control peptide did not selectively localize to the tumor. While the tumor to blood ratio in one mouse was 3.26, the control peptide distributed equally well to other organs (*e.g.* lung 3.52, spleen 3.27, liver 21.70, etc.). These results show that there was non-specific uptake of the control peptide in these organs. While abtide clone 14-DPTA-^{111}In demonstrated a tumor to blood ratio of only 1.85 at 2 hours (which appears lower than that obtained with the control peptide), clone 14-DPTA-^{111}In demonstrated specific localization to the tumor as the organ to blood ratios in the other organs were much lower (*e.g.* lung 0.81, spleen 0.83, liver 0.95, etc.).

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TABLE 5

**BIODISTRIBUTION OF GYK-DPTA-^{111}In
IN LNCaP XENOGRAFT BEARING MICE**

ORGAN/BLOOD	GROUP 1 MOUSE #		GROUP 2 MOUSE #		
	_1_	_2_	_3_	_4_	_5_
BLOOD	1.00	1.00	1.00	1.00	1.00
LUNG	2.96	3.52	0.95	2.84	2.39
SPLEEN	1.74	3.27	0.97	2.21	4.04
LIVER-S	31.79	21.70	21.56	25.38	17.78
KIDNEY-R	563.87	406.27	273.82	509.53	269.30
KIDNEY-L	584.69	417.98	297.65	433.97	280.50
TUMOR-S	1.14	3.26	0.86	1.68	1.87
MUSCLE	1.04	1.02	0.29	4.34	2.51
TESTES-R	603.01	58.49	0.82	2.05	2.25
TESTES-L	44.65	2.08	0.93	2.11	2.32

6.2. ABTIDES BINDING TO A BREAST CANCER ANTIGEN

6.2.1. IDENTIFICATION AND ISOLATION OF ABTIDES BINDING TO POLYMORPHIC EPITHELIAL MUCIN (PEM)

The monoclonal antibody SM-3 that specifically binds the polymorphic epithelial mucin (PEM) tumor antigen found on human breast cancer cells has been shown to be specific for the epitope defined by the amino acid sequence VTSAPDTRPAPGSTAPPAHGVTSAPDTR (SEQ ID NO: 9) (Bruchell et al., 1989, Int. J. Cancer 44:691-696). A peptide comprising this sequence was synthesized and used to isolate abtides from TSAR

peptide libraries by methods analogous to those described above. In these experiments, the specific TSAR libraries used were R26, D38 and DC43. See Figures 10-12 for description of these libraries. Phage bound to PEM were eluted by either

5 standard acid elution methods, stringent acid elution methods where phage were incubated with the PEM peptide for only 10 minutes prior to washing and elution, or were eluted using excess PEM peptide. Phage from each library were isolated that express peptides capable of binding to PEM. The amino

10 acid sequences of PEM binding phage are shown in Table 6.

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TABLE 6

Sequences of PEM Binding Phage

Acid Eluted

R26 Library

5

A15	SFMDYFFHTPEPK**PA**GYPNAYTDPKHPA	(SEQ ID NO: 26)
A54	SSSIFDYAPFSWG**SA**GLSNSSINVFERS	(SEQ ID NO: 27)
A5	SASLWDALGGWTTSAVPSYPRPHQTPGR	(SEQ ID NO: 28)
A39	SLGLPWIDVFGR**SS**AEPWPFGRTNLPRS	(SEQ ID NO: 29)
10 **A16**	SVHGAFLDSFFPW**AA**DGPHGRGRL-TSF	(SEQ ID NO: 30)

DC43 Library

15

MA-8	EEKQGGRWSTMMPRPWCHEG**GCG**FLYYDAMTKPKTPPIMRTAA	(SEQ ID NO: 31)
MA-21	LPRPFDDASWKLRAVKESPD**GCG**FGSPLLFPPYSGLPTFSSCD	(SEQ ID NO: 32)
20 **V22**	GSFESARGVTCIGNHSIGAH**GCG**PLRSYASFNRGSGRRH	(SEQ ID NO: 33)

D38 Library

25

MA-32	DQIGSRPQTTSRSISGSWWENAKTLWQQDYAFSAPNAA	(SEQ ID NO: 34)
V23	LSDAWGNFTTSYRDSAGFPSHAMTTSQGGKRNHASRFP	(SEQ ID NO: 35)
30 **V21**	VQLDDTSPRASGQETSQSEYDARPLLSKFAIPRPWSR	(SEQ ID NO: 36)
V1	IDSSKNRISGTGYLSFPHIRHANRRHMADDSNLAPGPS	(SEQ ID NO: 37)

35

Table 6 (continued)

Sequences of PEM Binding Phage

Acid Eluted: "Stringent"

DC43 Library

5

V44   WSIGTHTGPEGKFRIPCDRS**GCG**GTTLTHGGLNSSPTGQHERP   (SEQ ID
NO: 38)

10 **V39**   DPCEDGYWLSSVGRAGASIR**GCG**AIRRSSRTLTAEYSTRASNH   (SEQ ID
NO: 39)

V10   GSKRSCWGTTISNYFRPVPE**GCG**SASSINPNTNTGRLPSLHRQ   (SEQ ID
NO: 40)

V7   SSASSGCLGRAEHLDLDSVW**GCG**SQADMSRRYSPWYGRPRTGV   (SEQ ID
15   NO: 41)

V4   NVMWSSSKAGIRDCSQVPPG**GCG**PVNRHRASPPLTPFRHGSIR   (SEQ ID
NO: 42)

D38 Library

20

V45   PLTSGSSSEYRNRDDCPVYKYATNCPRLNFSPSRYSPF   (SEQ ID
NO: 43)

25 **V32**   GDAYGGIFSRPRQGLADSYIHASYTGKHFFRGPRPPTR   (SEQ ID
NO: 44)

V27   STCIGAEGEWKSFHNFLQCRDATSTSSSTLDPTALRFG   (SEQ ID
NO: 45)

V40   YSATLWDQFGSRQVELWSNRHASSALPFASRASVLGSR   (SEQ ID
30   NO: 46)

Peptide Eluted

R26 Library

35

P24   ILGWPFLTGLGDSTVHPRGRKGTDPS (SEQ ID NO: 47)

P49   SIPSFSMWLNQLGSAALPSKGNSQDRSD (SEQ ID NO: 48)

P26   SRDDIFTGGPLVLFRGSKTSNHDVHSMR (SEQ ID NO: 49)

P6   RAELVNWYEWFHVTAEAETPVINSHNMT (SEQ ID NO: 50)

Table 6 (continued)
Sequences of PEM Binding Phage

DC43 Library

5

MP-1	GAPVWRGNPRWRGPGGFKW**PGCG**NGPMCNTFTPARGGSRNNGP (SEQ ID NO: 51)
MP-2	GSASSCFPNFTARGVTVGFF**GCG**SPAHPAAPRVLNPATDFPAP (SEQ ID NO: 52)
MP-22	VFRRTARSSRPIGATVFPWY**GCG**NSNDETLPHHDSPPSFFLGA (SEQ ID NO: 53)
MA-13	NTCWTDLFWHGLPGGDLPRD**GCG**LPSELTTHPSRERRDASEN (SEQ ID NO: 54)

15

D38 Library

MP-20	IDWNWLERGQHNRGYLHSFPDAKSQPTRGPRVAPNGND (SEQ ID NO: 55)
MP-30	GRGSDMREHWPWSMPLILDQHANDPSPRAQSHYYSHPF (SEQ ID NO: 56)

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PENY2-379605.1

6.2.2. SATURATION MUTAGENESIS OF MP-1 AND IDENTIFICATION OF ADDITIONAL PEM BINDING ABTIDES

5   A saturation mutagenesis library based on one of the PEM abtides, MP-1, was constructed.   Nucleotide sequences encoding the MP-1 abtide were synthesized using a doping scheme similar to that described in Section 5.3 whereby each nucleotide was contaminated with 9% of each of the other 3 nucleotides
10 (e.g. G= 73% G, 9% A, 9% T, 9% C).   The resulting mutagenic oligonucleotides were used to construct a library by TSAR library methods described above (see Figure 13).

    The resulting library was screened to identify phage expressing abtides capable of binding to PEM.   The binding of
15 isolated phage to PEM was confirmed by an ELISA assay.   Phage that were shown to bind to PEM as well as phage that did not bind to PEM were sequenced to determine the amino acid sequences of the expressed abtides.   Table 7 shows the amino acid sequences of these positive and negative binding phage.

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Table 7

Sequence Comparison: MP-1 Binding Motif

Positive Binding Sequences

MP1   GAPVWRGNPRWRGPGGFKWPGCGNGPMCNTFTPARGGSRNNGP (SEQ ID NO: 51)

5

E4   VSTGWSGTPRWCAPGGKQGSGCGNGPRWTTLTPDLGGTRKYGP (SEQ ID NO: 57)

E7   GAPLWCEKLSGTGSGGFKWPGCGSGPTYNTFTPARVGSDNKWP (SEQ ID NO: 58)

E16   GPPVWSAKSRWTGTGVLNWPGCGKVPSCSTYTPSRDRSRKSDP (SEQ ID NO: 59)

E21   GSALLTSKGCVRGPGGLMRPGCGNDRLGKSSTYAHGGWIKTGP (SEQ ID NO: 60)

10 E33   GSPVWSGDNRWRGSSPLKRPGCGNGAKCNTLKDNRKDSRKTKH (SEQ ID NO: 61)

E44   G PLLPGEAAVHGARGLMRSGCGNGPTWNRLTAACRDSRNKGP (SEQ ID NO: 62)

E65   GSPVWMGSTRWTGHGWFRSQGCGNVPRTNSCAPAGKDSQNKGP (SEQ ID NO: 63)

E73   GAPVWRGNRWCSDNGELERPGCGYGPRFNILPPGRGNSRKPSP (SEQ ID NO: 64)

E84   GSSGWKVKHRCGGPGTLQRPGCGNLPLGHTFPPTRGGSHMEGA (SEQ ID NO: 65)

15 E85   GPRSWMGQPRGSDAGSCKWAGCGDAPMWRASTPGHGGPPNRGS (SEQ ID NO: 66)

E88   EALVCRGKPPWSGPAGLLWQGCGTGPVSRTFTSAQGRSRNKTS (SEQ ID NO: 67)

E90   GAPVVGDILWCSGARGAKWPGCGKGPTNKTFSHSRGGTQKSGL (SEQ ID NO: 68)

E22   GAPVSRCKPACGGFWGVNWPGCGNASMCKTFTNGHGVSSDNGH (SEQ ID NO: 69)

E29   GAHGYKNGSTCTGLGGWRCRGCGKGAMCNNPSPAGGAYHNQGP (SEQ ID NO: 70)

20 E62   G PQGSEHQCCSGHWGLKFPGCGNGPICNNFTALRGASRKNGP (SEQ ID NO: 71)

E64   GEPVWCRHSGGRVQGGLDWLGCGDGPLRYTVTPARGGPSKHGP (SEQ ID NO: 72)

E66   GLSLVRGDSWGSGAGGWKRHGCGHGPMYNPQTPARGGSCTRNT (SEQ ID NO: 73)

E67   VSRAWSGKPRLMGSHGLNCPGCGKGHSGIMFIPDPAGSANTPP (SEQ ID NO: 74)

E68   CAPMWSGKPPWCVGGGVKFRGCGNRPDCNIITPRLVESRDKAL (SEQ ID NO: 75)

25 E70   ADPVCSRKPDGGGLRGLRWPGCGKGPILYNVTAARGGSRNNGP (SEQ ID NO: 76)

Negative Binding Sequences

MPI   GAPVWRGNPRWRGPGGFKWPGCGNGPMCNTFTPARGGSRNNGP (SEQ ID NO: 51)

30 E3   GTRVPPGFALRGGRDGLSWAGCGKAPISKTYTSARGRSRKKGS (SEQ ID NO: 77)

E15   RSAVSEGKPREIVPGGCMWPGCGNGRKSNTLTHGPEQFQEIEP (SEQ ID NO: 78)

E24   SSGVGNGKPRSWAPDALNGGCGNIQFANTITPDRGGSCNQTL (SEQ ID NO: 79)

E27   GSSVCGGQPSGRGFGGLPGPGCGNGPTSNTLTSARGGFPNKGL (SEQ ID NO: 80)

E37   GAPLWQGDPADEVLGGSMIPGCGIGALSQTFTPTPGGSRKNVT (SEQ ID NO: 81)

35 E43   AGRELRQDEGEGGAGADVARLREGPICSTFTPARGGSCPSGL (SEQ ID NO: 82)

E49   QARVSMAISCRSGPSDLMHQGCGYGPRCNPDTTDSGGSHTNTP (SEQ ID NO: 83)

E60   GDPECRGKPRGRWTGSLACTGCGNGPNSKICTRARGVSRNKGP (SEQ ID NO: 84)

E72   STPGCSGYSGSGDPRCLTCTACGNGHTRKTLTPAHGRSTHKEP (SEQ ID NO: 85)

E34   GQPECRITSGCCGTDGNKWLGCGKVDMCNTLNPAVGCHGTNGS (SEQ ID NO: 86)

E83   REPVVGGKPWCRGPGGLRWRGCGKSQFDKIITLSRDNRRDKRP (SEQ ID NO: 87)

              PENY2-379605.1

When the sequences shown in Table 7 are compared (see particularly the amino acid residues marked in boldface type), it is possible to determine the influence of particular amino acid residues at specific positions in the sequence on a peptide's ability to bind to PEM. Abtides that bind to PEM can be characterized by the formula:

$R_1R_2R_3R_4R_5R_6R_7R_8R_9R_9R_{10}R_{11}R_{12}R_{13}R_{14}R_{15}R_{16}R_{17}R_{18}R_{19}R_{20}R_{21}R_{22}R_{23}R_{24}R_{25}R_{26}R_{27}R_{28}R_{29}$
$R_{30}R_{31}R_{32}R_{33}R_{34}R_{35}R_{36}R_{37}R_{38}R_{39}R_{40}R_{41}R_{42}R_{43}$ (SEQ ID NO: 88)

where:

R_1= G, C, E, or V, preferably G;
R_2= A, S, P, or L, preferably A;
R_3= P, T, H, or L, preferably P;
R_4= L, M, Q, G, A, or S;
R_5= W or Y, preferably W;
R_6= S, C, K or T, preferably S;
R_7= E, S, C, D, V, or R;
R_8= N, H, K, S, or E;
R_9= L, H, R, N, Q, T, or G;
R_{10}= W, P, R, T, or D, preferably W;
R_{11}= W, C, V, L, or G, preferably W;
R_{12}= S, T, M, or H, preferably S or T;
R_{13}= G;
R_{14}= S, A, G, N, Q, or H, preferably S;
R_{15}= W, H, G, A, or R;
R_{16}= G, T, E, P, V, or W, preferably G;
R_{17}= V, F, W, K, or A;
R_{18}= K, Q, D, E, R, or L, preferably K;
R_{19}= R, F, or S, preferably R;
R_{20}= P, S, I or H, preferably P;
R_{21}= G;
R_{22}= C;
R_{23}= G;
R_{24}= D, S, T, N, or H;
R_{25}= G, D, L, or R;
R_{26}= P or S, preferably P;
R_{27}= M, S, D, I, L, or R;
R_{28}= G, W, C, L, F, Y, or T, preferably G or W;

R_{29}= S, N, V, F, H, or R;

R_{30}= N, A, S, M, or R, preferably N;

R_{31}= F, Q, P, or V, preferably F;

R_{32}= S, V, I, K, A, or S;

5 R_{33}= P, A, N, or Y, preferably P;

R_{34}= G, N, or L;

R_{35}= K, R, C, Q or L, preferably K or R;

R_{36}= V, K, R, or A;

R_{37}= G, D, A, or E, preferably G;

10 R_{38}= S, T, P, Y or W; preferably S;

R_{39}= R, I, L, P, A or S;

R_{40}= N, K, or M, preferably N or K;

R_{41}= S, R, T, E, Q, P, Y or H;

R_{42}= G, A, S, D, N, P, Y, or K, preferably G;

15 R_{43}= P, H or A.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described 20 herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures 25 of which are incorporated by reference in their entireties.

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1. A molecule comprising a peptide which binds to a substance of interest, which peptide is identified by a method comprising:

    (a)   screening a first random peptide library with a first ligand, said first ligand being a specific binding partner of said substance of interest, to identify a first peptide that specifically binds to said first ligand; and

    (b)   screening a second random peptide library with a second ligand comprising said first peptide identified in step (a), to identify a second peptide which binds to said second ligand and which binds to said substance of interest.

2. A molecule comprising a peptide which binds to an antigen of interest, which peptide is identified by a method comprising:

    (a)   screening a first random peptide library with an antibody or antigen-binding derivative thereof that specifically binds to an antigen of interest, to identify a first peptide that specifically binds to said antibody or antigen-binding derivative thereof; and

    (b)   screening a second random peptide library with a compound comprising said first peptide identified in step (a), to identify a second peptide which binds to said compound and which binds to said antigen of interest.

3. The molecule of claim 2, in which said first random peptide library is a different library from said second random peptide library.

4. The molecule of claim 2, in which said first random peptide library is the same library as said second random peptide library.

5. The molecule of claim 1, in which said method further comprises comparing the sequences of a plurality of different first peptides identified as binding said first ligand in step (a), to identify a consensus binding sequence, in which said second ligand of step (b) comprises said consensus binding sequence.

6. The molecule of claim 2, in which said method further comprises comparing the sequences of a plurality of different first peptides identified as binding said antibody or antigen-binding derivative thereof in step (a), to identify a consensus binding sequence, in which said compound of step (b) comprises said consensus binding sequence.

7. The molecule of claim 1 in which the first ligand comprises a receptor.

8. The molecule of claim 2 in which the antibody is the monoclonal antibody 7E11-C5.

9. The molecule of claim 1 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

10. The molecule of claim 2 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more

contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

5   11.   The molecule of claim 1 in which the library of step (a) or step (b) is a chemically synthesized library.

10   12.   A molecule comprising: an amino acid sequence selected from the group consisting of:
    GIINANDPLPFWFMSPYTPGPAPIDINASRALVSNESG (SEQ ID NO: 1),
    CGRAYCLSGNYNIFGALFPGVSTPYADVGHDDAQSWRR (SEQ ID NO: 3),
    DLSRNLDFGRFLLYNAYVPGFTPTFISLTAEHLSSPKG (SEQ ID NO: 2),
    RCSPIWGISYPFGLLSSNPGVCHSSDAETNIRNDILTT (SEQ ID NO: 4),
15   and
    GHSNYCFVSTLGMPIVGFPSINARGLIHYGGSDPRLAA (SEQ ID NO: 5);
    or a binding portion thereof.

    13.   A peptide in which the amino acid sequence of
20   said peptide consists of the sequence selected from the group consisting of:
    GIINANDPLPFWFMSPYTPGPAPIDINASRALVSNESG (SEQ ID NO: 1),
    CGRAYCLSGNYNIFGALFPGVSTPYADVGHDDAQSWRR (SEQ ID NO: 3),
    DLSRNLDFGRFLLYNAYVPGFTPTFISLTAEHLSSPKG (SEQ ID NO: 2),
25   RCSPIWGISYPFGLLSSNPGVCHSSDAETNIRNDILTT (SEQ ID NO: 4),
    and
    GHSNYCFVSTLGMPIVGFPSINARGLIHYGGSDPRLAA (SEQ ID NO: 5);
    or a binding portion thereof.

30   14.   A method of identifying a peptide which binds to a substance of interest, comprising:

    (a)   screening a first random peptide library with a ligand, said ligand being a specific binding partner of said substance of
35   interest, to identify a first peptide that specifically binds to said ligand; and

    (b)   screening a second random peptide library with a compound comprising said first peptide identified in step (a), to identify a second peptide which binds to said

compound and which binds to said substance of interest.

15. A method of identifying a peptide which binds to an antigen of interest, comprising:

(a) screening a first random peptide library with an antibody or antigen-binding derivative thereof that specifically binds to an antigen of interest, to identify a first peptide that specifically binds to said antibody or antigen-binding derivative thereof; and

(b) screening a second random peptide library with a molecule comprising said first peptide identified in step (a), to identify a second peptide sequence which binds to said molecule and which binds to said antigen of interest.

16. The method of claim 14, in which said first random peptide library is a different library from said second random peptide library.

17. The method of claim 14, in which said first random peptide library is the same library as said second random peptide library.

18. The method of claim 14 in which the ligand is a receptor.

19. The method of claim 15 in which the antibody is the monoclonal antibody 7E11-C5.

20. The method of claim 14 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one

or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

5   21.   The method of claim 15 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an

10   oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

15

22.   The method of claim 14 where the library of step (a) or step (b) is a chemically synthesized library.

20   23.   A method of detecting or measuring an analyte of interest in a sample, comprising:

(a)   contacting a sample with a molecule comprising a peptide capable of specifically binding said analyte of

25   interest under conditions such that specific binding between said molecule and said analyte can occur; and

(b)   detecting or measuring the amount of

30   said binding in which the presence and amount of said binding indicates the presence and amount, respectively, of said analyte in the sample;

35   in which said peptide is identified by the method of claim 14.

24.   The method of claim 23 in which said molecule is immobilized on a solid substratum.

25.   A method of determining the location in
a patient of a tumor comprising:

(a)   introducing a molecule comprising a
        peptide that specifically binds to
        a tumor antigen into the patient;
        and

(b)   determining the location in the
        patient of the molecule;

in which the molecule is detectably labeled; and in
which said peptide is identified by a method comprising:

(i)   screening a first random
        peptide library with an
        antibody or antigen-
        binding derivative
        t h e r e o f   t h a t
        specifically binds to
        said tumor antigen, to
        identify a first peptide
        that specifically binds
        to said antibody or
        a n t i g e n - b i n d i n g
        derivative thereof; and

(ii)   screening a second
        random peptide library
        with a molecule
        comprising said first
        peptide identified in
        (i), to identify a
        second peptide which
        binds to said molecule
        and which binds to said
        tumor antigen.

26.   A therapeutic or diagnostic composition
comprising the molecule of claim 1; and a
pharmaceutically acceptable carrier.

27.   A therapeutic or diagnostic composition
comprising the molecule of claim 2; and a
pharmaceutically acceptable carrier.

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28. A therapeutic or diagnostic composition comprising the molecule of claim 5; and a pharmaceutically acceptable carrier.

29. A therapeutic or diagnostic composition comprising the molecule of claim 7; and a pharmaceutically acceptable carrier.

30. A therapeutic or diagnostic composition comprising the molecule of claim 8; and a pharmaceutically acceptable carrier.

31. A therapeutic or diagnostic composition comprising the molecule of claim 12; and a pharmaceutically acceptable carrier.

32. A composition comprising a plurality of molecules of claim 1, in which said peptide sequences of said molecules differ.

33. A molecule comprising a peptide or a binding portion thereof which binds to a ligand of interest, which peptide is identified by a method comprising: screening a random peptide library with a ligand of interest, said ligand of interest being a peptide having a length of between 5 and 40 amino acids, to identify a peptide that specifically binds to the ligand of interest, in which the ligand of interest is also specifically bound by an antibody or a receptor.

34. The molecule of claim 31 in which the ligand is a peptide having a length of between 10 and 20 amino acids.

35. A method of obtaining an image of an internal region of a subject comprising administering to said subject an effective amount of the molecule of claim 1 in which said molecule is radiolabeled with a radioactive metal, and recording the scintigraphic image obtained from the decay of said radioactive metal.

36. A molecule comprising a peptide which binds to a substance of interest, which peptide is identified by a method comprising: screening a random peptide library with a ligand, said ligand being a peptide of 36 amino acids or fewer, in which the ligand is an epitope of an antigen that is specifically bound by an antibody or in which the ligand represents the portion of a receptor-ligand that is responsible for the specific binding of the receptor to the receptor-ligand.

37. A peptide comprising the amino acid sequence WQGTHF (SEQ ID NO: 23) and the amino acid sequence LVSKNDSG (SEQ ID NO: 24) that specifically binds to an antigen of human prostate carcinoma cells.

38. A molecule comprising an amino acid sequence selected from the group consisting of:

SFMDYFFHTPEPKPAGYPNAYTDPKHPA (SEQ ID NO: 26),

SSSIFDYAPFSWGSAGLSNSSINVFERS (SEQ ID NO: 27),

SASLWDALGGWTTSAVPSYPRPHQTPGR (SEQ ID NO: 28),

SLGLPWIDVFGRSSAEPWPFGRTNLPRS (SEQ ID NO: 29),

SVHGAFLDSFFPWAADGPHGRGRLTSF (SEQ ID NO: 30),

EEKQGGRWSTMMPRPWCHEGGCGFLYYDAMTKPKTPPIMRTAA (SEQ ID NO: 31),

LPRPFDDASWKLRAVKESPDGCGFGSPLLFPPYSGLPTFSSCD (SEQ ID NO: 32),

GSFESARGVTCIGNHSIGAHGCGPLRSYASFNRGSGRRH (SEQ ID NO: 33),

DQIGSRPQTTSRSISGSWWENAKTLWQQDYAFSAPNAA (SEQ ID NO: 34),

LSDAWGNFTTSYRDSAGFPSHAMTTSQGGKRNHASRFP (SEQ ID NO: 35),

VQLDDTSPRASGQETSQSEYDARPLLSKFAIPRPWSR (SEQ ID NO: 36),

IDSSKNRISGTGYLSFPHIRHANRRHMADDSNLAPGPS (SEQ ID NO: 37),

WSIGTHTGPEGKFRIPCDRSGCGGTTLTHGGLNSSPTGQHERP (SEQ ID NO: 38),

DPCEDGYWLSSVGRAGASIRGCGAIRRSSRTLTAEYSTRASNH (SEQ ID NO: 39),

GSKRSCWGTTISNYFRPVPEGCGSASSINPNTNTGRLPSLHRQ
(SEQ ID NO: 40),
SSASSGCLGRAEHLDLDSVWGCGSQADMSRRYSPWYGRPRTGV
(SEQ ID NO: 41),
NVMWSSSKAGIRDCSQVPPGGCGPVNRHRASPPLTPFRHGSIR
(SEQ ID NO: 42),
PLTSGSSSEYRNRDDCPVYKYATNCPRLNFSPSRYSPF (SEQ ID
NO: 43),
GDAYGGIFSRPRQGLADSYIHASYTGKHFFRGPRPPTR (SEQ ID
NO: 44),
STCIGAEGEWKSFHNFLQCRDATSTSSSTLDPTALRFG (SEQ ID
NO: 45),
YSATLWDQFGSRQVELWSNRHASSALPFASRASVLGSR (SEQ ID
NO: 46),
ILGWPFLTGLGDSTVHPRGRKGTDPS (SEQ ID NO: 47),
SIPSFSMWLNQLGSAALPSKGNSQDRSD (SEQ ID NO: 48),
SRDDIFTGGPLVLFRGSKTSNHDVHSMR (SEQ ID NO: 49),
RAELVNWYEWFHVTAEAETPVINSHNMT (SEQ ID NO: 50),
GAPVWRGNPRWRGPGGFKWPGCGNGPMCNTFTPARGGSRNNGP
(SEQ ID NO: 51),
GSASSCFPNFTARGVTVGFFGCGSPAHPAAPRVLNPATDFPAP
(SEQ ID NO: 52),
VFRRTARSSRPIGATVFPWYGCGNSNDETLPHHDSPPSFFLGA
(SEQ ID NO: 53),
NTCWTDLFWHGLPGGDLPRDGCGLPSELTTHPSRERRDASEN
(SEQ ID NO: 54),
IDWNWLERGQHNRGYLHSFPDAKSQPTRGPRVAPNGND (SEQ ID
NO: 55) and
GRGSDMREHWPWSMPLILDQHANDPSPRAQSHYYSHPF (SEQ ID
NO: 56).

39.   A molecule comprising an amino acid
sequence selected from the group consisting of:
VSTGWSGTPRWCAPGGKQGSGCGNGPRWTTLTPDLGGTRKYGP (SEQ ID
NO: 57),
GAPLWCEKLSGTGSGGFKWPGCGSGPTYNTFTPARVGSDNKWP (SEQ ID
NO: 58),
GPPVWSAKSRWTGTGVLNWPGCGKVPSCSTYTPSRDRSRKSDP (SEQ ID
NO: 59),

GSALLTSKGCVRGPGGLMRPGCGNDRLGKSSTYAHGGWIKTGP (SEQ ID NO: 60),

GSPVWSGDNRWRGSSPLKRPGCGNGAKCNTLKDNRKDSRKTKH (SEQ ID NO: 61),

G PLLPGEAAVHGARGLMRSGCGNGPTWNRLTAACRDSRNKGP (SEQ ID NO: 62),

GSPVWMGSTRWTGHGWFRSQGCGNVPRTNSCAPAGKDSQNKGP (SEQ ID NO: 63),

GAPVWRGNRWCSDNGELERPGCYGPRFNILPPGRGNSRKPSP (SEQ ID NO: 64),

GSSGWKVKHRCGGPGTLQRPGCGNLPLGHTFPPTRGGSHMEGA (SEQ ID NO: 65),

GPRSWMGQPRGSDAGSCKWAGCGDAPMWRASTPGHGGPPNRGS (SEQ ID NO: 66),

EALVCRGKPPWSGPAGLLWQGCGTGPVSRTFTSAQGRSRNKTS (SEQ ID NO: 67),

GAPVVGDILWCSGARGAKWPGCGKGPTNKTFSHSRGGTQKSGL (SEQ ID NO: 68),

GAPVSRCKPACGGFWGVNWPGCGNASMCKTFTNGHGVSSDNGH (SEQ ID NO: 69),

GAHGYKNGSTCTGLGGWRCRGCGKGAMCNNPSPAGGAYHNQGP (SEQ ID NO: 70),

G PQGSEHQCCSGHWGLKFPGCGNGPICNNFTALRGASRKNGP (SEQ ID NO: 71),

GEPVWCRHSGGRVQGGLDWLGCGDGPLRYTVTPARGGPSKHGP (SEQ ID NO: 72),

GLSLVRGDSWGSGAGGWKRHGCGHGPMYNPQTPARGGSCTRNT (SEQ ID NO: 73),

VSRAWSGKPRLMGSHGLNCPGCGKGHSGIMFIPDPAGSANTPP (SEQ ID NO: 74),

CAPMWSGKPPWCVGGGVKFRGCGNRPDCNIITPRLVESRDKAL (SEQ ID NO: 75) and

ADPVCSRKPDGGGLRGLRWPGCGKGPILYNVTAARGGSRNNGP (SEQ ID NO: 76).

40. The molecule which binds to a ligand of interest of claim 33 in which said ligand comprises VTSAPDTRPAPGSTAPPAHGVTSAPDTR (SEQ ID NO: 9) or a portion thereof.

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41. A therapeutic or diagnostic composition comprising a molecule chosen from the group of molecules of claim 38 and a pharmaceutically acceptable carrier.

42. A therapeutic or diagnostic composition comprising a molecule chosen from the group of molecules of claim 39 and a pharmaceutically acceptable carrier.

43. A molecule that binds to polymorphic epithelial mucin, comprising an amino acid sequence represented by the formula:

$R_1R_2R_3R_4R_5R_6R_7R_8R_9R_9R_{10}R_{11}R_{12}R_{13}R_{14}R_{15}R_{16}R_{17}R_{18}R_{19}R_{20}R_{21}R_{22}R_{23}R_{24}R_{25}R_{26}$
$R_{27}R_{28}R_{29}R_{30}R_{31}R_{32}R_{33}R_{34}R_{35}R_{36}R_{37}R_{38}R_{39}R_{40}R_{41}R_{42}R_{43}$ (SEQ ID NO: 88)

wherein:

R_1= G, C, E, or V;

R_2= A, S, P, or L;

R_3= P, T, H, or L;

R_4= L, M, Q, G, A, or S;

R_5= W or Y;

R_6= S, C, K or T;

R_7= E, S, C, D, V, or R;

R_8= N, H, K, S, or E;

R_9= L, H, R, N, Q, T, or G;

R_{10}= W, P, R, T, or D;

R_{11}= W, C, V, L, or G;

R_{12}= S, T, M, or H;

R_{13}= G;

R_{14}= S, A, G, N, Q, or H;

R_{15}= W, H, G, A, or R;

R_{16}= G, T, E, P, V, or W;

R_{17}= V, F, W, K, or A;

R_{18}= K, Q, D, E, R, or L;

R_{19}= R, F, or S;

R_{20}= P, S, I or H;

R_{21}= G;

R_{22}= C;

R_{23}= G;

R_{24}= D, S, T, N;

R_{25}= G, D, L;

R_{26}= P or S;

R_{27}= M, S, D, I, L, or R;

R_{28}= G, W, C, L, F, Y, or T;

5 R_{29}= S, N, V, F, H, or R;

R_{30}= N, A, S, M, or R;

R_{31}= F, Q, P, or V;

R_{32}= S, V, I, K, A, or S;

R_{33}= P, A, N, or Y;

10 R_{34}= G, N, or L;

R_{35}= K, R, C, Q or L;

R_{36}= V, K, R, or A;

R_{37}= G, D, A, or E;

R_{38}= S, T, P, Y or W;

15 R_{39}= R, I, L, P, A or S;

R_{40}= N, K, or M;

R_{41}= S, R, T, E, Q, P, Y or H;

R_{42}= G, A, S, D, N, P, Y, or K;

R_{43}= P, H or A.

20

44. The molecule of claim 43 wherein:

R_{1}= G;

R_{2}= A;

R_{3}= P;

25 R_{5}= W;

R_{6}= S;

R_{10}= W;

R_{11}= W;

R_{12}= S or T;

30 R_{14}= S;

R_{16}= G;

R_{18}= K;

R_{19}= R;

R_{20}= P;

35 R_{26}= P;

R_{28}= G or W;

R_{30}= N;

R_{31}= F;

R_{33}= P;

R_{35}= K or R;

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$R_{37}= G$;
$R_{38}= S$;
$R_{40}= N$ or K;
$R_{42}= G$;

5

45. The molecule of claim 2 in which the antibody or antigen-binding derivative thereof is capable of specifically binding to a human tumor

10   antigen.

15

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25

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ABSTRACT

Abtides are provided. Abtides are peptides identified by a two-step process of screening random peptide libraries. In the first step, the target ligand is an antibody or receptor (or derivative thereof). The peptides identified in the first screening step are used as target ligands in the second screening step. The peptides identified in the second screening step are abtides. Abtides possess binding specificities that are similar to the binding specificities of the antibodies or receptors that are used in the first screening step. Abtides may be used in place of antibodies in many assays or therapeutic applications.

Abtides binding to polymorphic epithelial mucin (PEM) are provided.

Also provided are methods of obtaining abtides as well as diagnostic and therapeutic compounds containing abtides.

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FIG.1

FIG.2

OD (405 nm)

ANTIBODY CONCENTRATION (μM)

CLONE 14   GIINANDPLPFWFMS--PYTPGPAPIDINASRALVS-NESG
                  WQGTHFPYT   LVSKNDSG
                   CDR3L   CDR2L
            (5/9 = 55.5 %)   (7/8 = 87.5 %)

CLONE 17   DL-SRNLDFGRFLLYNA--YVPGFTPTFISLTAEHLSSPKG
         LVSKN-DSG   WQGTHF-P-YT
           CDR2L   CDR3L
        (6/8 = 75 %)   (6/9 = 66.6 %)

CLONE 15   CGRAYCL-SGNYNIFGALFPGVS--TPYADVGHDDAQSWRR
           LVSKN-DS-G WQG-THFPYT
            CDR2L   CDR3L
        (4/8 = 50 %) (6/9 = 66.6 %)

CLONE 13   RCSPIW-GIS-YPFGLLSSNPGVCHSSDAET-NIRNDILTT
          WQG-THFPYT   GSDN-K-SVL
           CDR3L   CDR2L(REV)
        (6/9 = 66.6 %)   (4/8 = 50 %)

CLONE 16   GHSNYCFVSTLGMPIVGFP-SINARGLIHYGGSDPR--LAA
               WQGTHFPYT   GSDNKSVL
                 CDR3L   CDR2L(REV)
           (3/9 = 33.3 %) (5/8 = 62.5 %)

FIG.3

FIG.4

FIG.5

PEPTIDE CONCENTRATION (μM)

FIG.6

FIG.7

FIG.8

ORGAN:BLOOD RATIO

20.00

15.00

10.00

5.00

0

BLOOD LUNG SPLEEN LIVER TUMOR MUSCLE

ORGAN

FIG.9

ORGAN:BLOOD

40.00

30.00

20.00

10.00

0

BLOOD LUNG SPLEEN LIVER TUMOR MUSCLE

ORGAN

Sac II

c.tgt.gcc.tcg.agB.(NNB)$_{12}$.Ncc.gcg.g

gg.cgc.cNV.(NNV)$_{12}$.aga.tct.cgt.gtc

N=A,G,T,C
B=G,T,C
V=G,A,C

FILL IN WITH DNA POLYMERASE

Xho I   Ala

c.tgt.gcc.tcg.agB.(NNB)$_{12}$.Ncc.gcg.g

gg.cgc.cNV.(NNV)$_{12}$.aga.tct.cgt.gtc

Xba I

CLEAVE WITH Xho I + Xba I

tcg.agB.(NNB)$_{12}$.Ncc.gcg.g

gg.cgc.cNV.(NNV)$_{12}$.tga.tc

LIGATE WITH Xho I + Xba I-
CLEAVED M13 m663 VECTOR

ELECTROPORATE INTO XL1-BLUE

LIBRARY OF pIII-RANDOM SEQUENCE FUSION PROTEINS

. . . S H S | S (S/R) X$_{12}$ π A ∂ X$_{12}$ S R | P S R T . . .

π=S,P,T OR A
∂=V,A,D,E, OR G

SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.10

G TGT GTC TCG AGN (NNB)$_{20}$NAC GCC AN
_____

                              _____
                              NTG CGG TNV (NNV)$_{15}$AGA TCT GTG TTG

N=A,C,G,T
B=C,G,T   ↓   FILL IN WITH SEQUENASE
V=A,C,G

          __Xho I__
G TGT GTC TCG AGN (NNB)$_{20}$NAC GCC AN
_____
=======================================

==
NTG CGG TNV (NNV)$_{15}$AGA TCT GTG TTG
                           ‾‾‾‾‾‾‾
                           Xba I

                              ↓   RESTRICT WITH Xho I AND Xba I

    TCG AGN (NNB)$_{20}$ NAC GCC AN
    _____

                     _____
                     NTG CGG TNV (NNV)$_{15}$AGA TC

                     ↓   LIGATE WITH Xho I + Xba I-
                     │   CLEAVED M13mp18Xa
                     │
                     ↓   ELECTROTRANSFORM
                          E. coli JS5

        D38 GENETIC DIVERSITY LIBRARY DISPLAYED AS RANDOM
        N-TERMINAL pIII FUSIONS

. . H S↑S (S/R) X$_{20}$ (Y/H/N/D) A (I/M/T/N/K/S/R) X$_{15}$ S R
      │
      │SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.11

G TGT GTC TCG AGN (NNB)$_{20}$GGT TGT GGT

CCA ACA CCA (NNV)$_{20}$AGA TCT GTG TTG

N=A,C,G,T
B=C,G,T
V=A,C,G

FILL IN WITH SEQUENASE

Xho I

G TGT GTC TCG AGN (NNB)$_{20}$GGT TGT GGT

CCA ACA CCA (NNV)$_{20}$AGA TCT GTG TTG
Xba I

RESTRICT WITH Xho I AND Xba I

TCG AGN (NNB)$_{20}$ GGT TGT GGT

CCA ACA CCA (NNV)$_{20}$AGA TC

LIGATE WITH Xho I + Xba I-
CLEAVED M13mp18Xa

ELECTROTRANSFORM
E. coli JS5

DC43 GENETIC DIVERSITY LIBRARY DISPLAYED AS RANDOM
N-TERMINAL pIII FUSIONS

. . H S S (S/R) X$_{20}$ G C G X$_{20}$ S R

SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.12

G A P V W R G N P R W R G P G G F K W P G C G N G P M C N T F T P A R G G S R N N G P

5' ggs-gcs-ccs-gts-tgs-ags-ggs-aas-ccs-cgs-tgs-ags-ggs-tts-aas-tgs-ccs-ggs-tgs-ggs-tts-aas-tgs-ccs-ggs-ggs-ccs-ggs-ggs-ttc-aas-tgs-ccs-ggs-tgc-ggs-aas-ggs 3'

MP-1 oligo 1

3' CCG-ACG-CCC-tts-ccs-ggs-tas-acs-tts-tgs-aas-tgs-ggs-tgs-ggs-ccs-ccs-ags-gcs-ccs-tts-ccs-ggs 5'

MP-1 oligo 2

FIG.13

DECLARATION
AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

ANTIGEN BINDING PEPTIDES (ABTIDES) FROM PEPTIDE LIBRARIES

the specification of which is attached hereto unless one of the following boxes is checked:
☒ the specification was filed in the United States on June 7, 1995_____ as Application Serial No. 08/488,161_____ *(for declaration not accompanying application)*
with amendment(s) filed on _____ *(if applicable)*

☐ the specification was filed as PCT international application Serial No. _____ on _____ and was amended under PCT Article 19 on _____ *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION				
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED	
			YES ☐	NO ☐
			YES ☐	NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED
08/310,192	September 21, 1994		X	

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Isaac Jarkovsky (Reg. No. 22713), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753), Jon R. Stark (Reg. No. 30111), Allan A. Fanucci (Reg. No. 30256), Geraldine F. Baldwin (Reg. No. 31232), Victor N. Balancia (Reg. No. 31231), Albert P. Halluin (Reg. No. 25227), and Marcia H. Sundeen (Reg. No. 30893), all of Pennie & Edmonds, whose addresses are 1155 Avenue of the Americas, New York, New York 10036, 1667 K Street N.W., Washington, DC 20006 and 2730 Sand Hill Road, Menlo Park, CA 94025, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

END CORRESPONDENCE TO:	PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, N.Y. 10036-2711	DIRECT TELEPHONE CALLS TO: PENNIE & EDMONDS DOCKETING (212) 790-2803

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	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY ZIP CODE

2 0 5	**FULL NAME OF INVENTOR**	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY ZIP CODE

2 0 6	**FULL NAME OF INVENTOR**	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY ZIP CODE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 VERNON L. ALVAREZ DATE 8/9/95	SIGNATURE OF INVENTOR 202 DATE	SIGNATURE OF INVENTOR 203 DATE
SIGNATURE OF INVENTOR 204 DATE	SIGNATURE OF INVENTOR 205 DATE	SIGNATURE OF INVENTOR 206 DATE

PENY2-392079.1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Alvarez, Vernon L.

    (ii) TITLE OF INVENTION: Antigen Binding Peptides (Abtides) From
           Peptide Libraries

   (iii) NUMBER OF SEQUENCES: 103

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Pennie & Edmonds
        (B) STREET: 1155 Avenue of the Americas
        (C) CITY: New York
        (D) STATE: New York
        (E) COUNTRY: USA
        (F) ZIP: 10036

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/488,161
        (B) FILING DATE: 07-JUN-1995
        (C) CLASSIFICATION:

  (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: Misrock, S. Leslie
        (B) REGISTRATION NUMBER: 18,872
        (C) REFERENCE/DOCKET NUMBER: 1101-176

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Gly Ile Ile Asn Ala Asn Asp Pro Leu Pro Phe Trp Phe Met Ser Pro
1               5                   10                  15

Tyr Thr Pro Gly Pro Ala Pro Ile Asp Ile Asn Ala Ser Arg Ala Leu
            20                  25                  30

Val Ser Asn Glu Ser Gly
            35
```

(2) INFORMATION FOR SEQ ID NO:2:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 38 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

    Asp Leu Ser Arg Asn Leu Asp Phe Gly Arg Phe Leu Leu Tyr Asn Ala
    1   5   10   15

    Tyr Val Pro Gly Phe Thr Pro Thr Phe Ile Ser Leu Thr Ala Glu His
                20   25   30

    Leu Ser Ser Pro Lys Gly
                35

(2) INFORMATION FOR SEQ ID NO:3:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 38 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

    Cys Gly Arg Ala Tyr Cys Leu Ser Gly Asn Tyr Asn Ile Phe Gly Ala
    1   5   10   15

    Leu Phe Pro Gly Val Ser Thr Pro Tyr Ala Asp Val Gly His Asp Asp
                20   25   30

    Ala Gln Ser Trp Arg Arg
                35

(2) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 38 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

    Arg Cys Ser Pro Ile Trp Gly Ile Ser Tyr Pro Phe Gly Leu Leu Ser
    1   5   10   15

    Ser Asn Pro Gly Val Cys His Ser Ser Asp Ala Glu Thr Asn Ile Arg
                20   25   30

```
         Asn Asp Ile Leu Thr Thr
                 35
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly His Ser Asn Tyr Cys Phe Val Ser Thr Leu Gly Met Pro Ile Val
1               5               10              15

Gly Phe Pro Ser Ile Asn Ala Arg Gly Leu Ile His Tyr Gly Gly Ser
            20              25              30

Asp Pro Arg Leu Ala Ala
            35
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Trp Gln Gly Thr His Phe Pro Tyr Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Leu Val Ser Lys Asn Asp Ser Gly
1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids

(B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

     Gly Ser Asp Asn Lys Ser Val Leu
     1   5

(2) INFORMATION FOR SEQ ID NO:9:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 28 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

     Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro
     1   5   10   15

     Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg
                    20   25

(2) INFORMATION FOR SEQ ID NO:10:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 6 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

     Met Tyr Xaa Xaa Leu His
     1   5

(2) INFORMATION FOR SEQ ID NO:11:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
            Ser Cys Val Ser His Met Leu Asp Thr Ser Arg Val Tyr Thr Ala Tyr
            1               5               10              15

            Ala Asn Pro Gly Met Tyr Ser Arg Leu His Ser Pro Ala Val Arg Pro
                        20              25              30

            Leu Thr Gln Ser Ser Ala
                    35
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
            Ser Val Gln Phe Lys Ser Ile Ser Ser Arg Ser Met Asp Asp Val Val
            1               5               10              15

            Lys Asp Pro Gly Pro Lys Pro Ala Met Tyr Asn Arg Leu His Ser Lys
                        20              25              30

            Asn Pro Phe Thr Leu Ser
                    35
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
            Tyr Phe Asp His Thr Tyr Ser Gly Pro Val Val Lys Asn Gly Gly Leu
            1               5               10              15

            Val Ser Pro Gly Val Leu Ser Met Tyr Asn Arg Leu His Ser Asp Gly
                        20              25              30

            Gly Pro Ser Leu Ala Ser
                    35
```

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Thr Val Ala Thr Met His Asp Arg Leu His Ser Ala Pro Gly Ser Gly
1               5                   10              15

Asn Leu Pro Gly Ser Tyr Asp Ile Lys Pro Ile Phe Lys Ala Gln Ser
            20                  25              30

Gly Ala Leu His Ser Thr
            35
```

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Ile Asp Met Pro Gln Thr Ala Ser Thr Met Tyr Asn Met Leu His Arg
1               5                   10              15

Asn Glu Pro Gly Gly Arg Lys Leu Ser Pro Pro Ala Asn Asp Met Pro
            20                  25              30

Pro Ala Leu Leu Lys Arg
            35
```

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Arg Leu Gly Asn His Val Trp Arg Glu Gly Gly Gly Met Tyr Gln Gln
1               5                   10              15

Leu His His Asn Phe Pro
            20
```

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Arg Asp Ser Ala Val Glu Asn Pro Ser Val Gly Gly Glu Ile Pro Met
1               5               10                  15

Tyr Arg Tyr Leu His Gln Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Pro Val Gln Lys Glu Tyr Gly Phe Gly Met Ser Gly Ala Ser Met Ile
1               5               10                  15

Arg Leu Leu Arg Glu Thr Pro
            20
```

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Gln Lys Gly Gly Pro Gly Leu Leu Leu Tyr Gly Gly Asp Ser Met Tyr
1               5               10                  15

Ile Thr Leu His Glu Pro Gly
            20
```

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Leu Tyr Ala Asn Pro Gly Met Tyr Ser Arg Leu His Ser Pro Ala
1               5               10              15
```

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Gly Met Tyr Ser Arg Leu His Ser Pro Ala
1               5               10
```

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 35 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
His Cys Pro Pro Thr Pro Glu Thr Ser Cys Ala Thr Gln Thr Ile Thr
1               5               10              15

Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp Phe Leu Leu Val Ile Pro
        20              25              30

Phe Asp Cys
        35
```

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Arg Glu Pro Val Val Gly Gly Lys Pro Trp Cys Arg Gly Pro Gly Gly
1               5               10              15

Leu Arg Trp Arg Gly Cys Gly Lys Ser Gln Phe Asp Lys Ile Ile Thr
        20              25              30

Leu Ser Arg Asp Asn Arg Arg Asp Lys Arg Pro
```

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 1
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Cys, Glu, or Val"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 2
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ala, Ser, Pro or Leu"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 3
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Pro, Thr, His or Leu"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 4
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Leu, Met, Gln, Gly, Ala, or Ser"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 5
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Trp or Tyr"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 6
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Cys, Lys or Thr"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 7
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Glu, Ser, Cys, Asp, Val, or Arg"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 8
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Asn, His, Lys, Ser or Glu"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 9
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Leu, His, Arg, Asn, Gln, Thr or Gly"

```
(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 10
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Trp, Pro, Arg, Thr, or Asp"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 11
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Trp, Cys, Val, Leu, or Gly"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 12
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Thr, Met, or His"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 13
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 14
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Ala, Gly, Asn, Gln, or His"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 15
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Trp, His, Gly, Ala, or Arg"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 16
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Thr, Glu, Pro, Val, or Trp"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 17
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Val, Phe, Trp, Lys, or Ala"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 18
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Lys, Gln, Asp, Glu, Arg, and Leu"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 19
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Arg, Phe, or Ser"

(ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 20
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Pro, Ser, Ile, or His"

(ix) FEATURE:
```

```
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 21
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 22
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Cys"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 23
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 24
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Asp, Ser, Thr, or Asn"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 25
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Asp, or Leu"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 26
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Pro or Ser"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 27
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Met, Ser, Asp, Ile, Leu, or Arg"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 28
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Trp, Cys, Leu, Phe, Tyr, or Thr"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 29
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Asn, Val, Phe, His or Arg"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 30
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Asn, Arg, Ser, Met, or Arg"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 31
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Phe, Gln, Pro, or Val"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
```

```
              (B) LOCATION: 32
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Val, Ile, Lys, Ala or Ser"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 33
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Pro, Ala, Asn, or Tyr"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 34
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Asn, or Leu"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 35
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Lys, Arg, Cys, Gln, or Leu"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 36
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Val, Lys, Arg, or Ala"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 37
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Asp, Ala, or Glu"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 38
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Thr, Pro, Tyr, or Trp"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 39
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Arg, Ile, Leu, Pro, Ala, or Ser"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 40
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Asn, Lys, or Met"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 41
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Arg, Thr, Glu, Gln, Pro, Tyr, or His"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 42
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Gly, Ala, Ser, Asp, Asn, Pro, Tyr, or Lys"

    (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 43
```

(D) OTHER INFORMATION: /label= Xaa
     /note= "Xaa = Pro, His, or Ala"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

     Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
     1   5   10   15

     Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                     20   25   30

     Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
             35   40

(2) INFORMATION FOR SEQ ID NO:25:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 31 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (ix) FEATURE:
            (A) NAME/KEY: Modified-site
            (B) LOCATION: 2
            (D) OTHER INFORMATION: /label= Xaa
     /note= "Xaa = Ser or Arg"

        (ix) FEATURE:
            (A) NAME/KEY: Modified-site
            (B) LOCATION: 15
            (D) OTHER INFORMATION: /label= Xaa
     /note= "Xaa = Ser, Pro, Thr, or Ala"

        (ix) FEATURE:
            (A) NAME/KEY: Modified-site
            (B) LOCATION: 17
            (D) OTHER INFORMATION: /label= Xaa
     /note= "Xaa = Val, Ala, Asp, Glu, or Gly"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

     Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala
     1   5   10   15

     Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Arg
                     20   25   30

(2) INFORMATION FOR SEQ ID NO:26:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 28 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

                              -91-

```
Ser Phe Met Asp Tyr Phe Phe His Thr Pro Glu Pro Lys Pro Ala Gly
1             5               10              15

Tyr Pro Asn Ala Tyr Thr Asp Pro Lys His Pro Ala
            20              25
```

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Ser Ser Ser Ile Phe Asp Tyr Ala Pro Phe Ser Trp Gly Ser Ala Gly
1             5               10              15

Leu Ser Asn Ser Ser Ile Asn Val Phe Glu Arg Ser
            20              25
```

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Ser Ala Ser Leu Trp Asp Ala Leu Gly Gly Trp Thr Thr Ser Ala Val
1             5               10              15

Pro Ser Tyr Pro Arg Pro His Gln Thr Pro Gly Arg
            20              25
```

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Ser Leu Gly Leu Pro Trp Ile Asp Val Phe Gly Arg Ser Ser Ala Glu
1             5               10              15

Pro Trp Pro Phe Gly Arg Thr Asn Leu Pro Arg Ser
```

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Ser Val His Gly Ala Phe Leu Asp Ser Phe Phe Pro Trp Ala Ala Asp
1               5               10              15

Gly Pro His Gly Arg Gly Arg Leu Thr Ser Phe
            20              25
```

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Glu Glu Lys Gln Gly Gly Arg Trp Ser Thr Met Met Pro Arg Pro Trp
1               5               10              15

Cys His Glu Gly Gly Cys Gly Phe Leu Tyr Tyr Asp Ala Met Thr Lys
            20              25              30

Pro Lys Thr Pro Pro Ile Met Arg Thr Ala Ala
        35              40
```

(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Leu Pro Arg Pro Phe Asp Asp Ala Ser Trp Lys Leu Arg Ala Val Lys
1               5               10              15

Glu Ser Pro Asp Gly Cys Gly Phe Gly Ser Pro Leu Leu Phe Pro Pro
            20              25              30
```

```
           Tyr Ser Gly Leu Pro Thr Phe Ser Ser Cys Asp
                    35                  40
```

(2) INFORMATION FOR SEQ ID NO:33:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 39 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Gly Ser Phe Glu Ser Ala Arg Gly Val Thr Cys Ile Gly Asn His Ser
1               5                   10                  15

Ile Gly Ala His Gly Cys Gly Pro Leu Arg Ser Tyr Ala Ser Phe Asn
            20                  25                  30

Arg Gly Ser Gly Arg Arg His
            35
```

(2) INFORMATION FOR SEQ ID NO:34:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Asp Gln Ile Gly Ser Arg Pro Gln Thr Thr Ser Arg Ser Ile Ser Gly
1               5                   10                  15

Ser Trp Trp Glu Asn Ala Lys Thr Leu Trp Gln Gln Asp Tyr Ala Phe
            20                  25                  30

Ser Ala Pro Asn Ala Ala
            35
```

(2) INFORMATION FOR SEQ ID NO:35:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Leu Ser Asp Ala Trp Gly Asn Phe Thr Thr Ser Tyr Arg Asp Ser Ala
```

1			5						10					15
Gly	Phe	Pro	Ser	His	Ala	Met	Thr	Thr	Ser	Gln	Gly	Gly	Lys	Arg	Asn

```
          20                        25                        30
His Ala Ser Arg Phe Pro
          35
```

(2) INFORMATION FOR SEQ ID NO:36:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Val Gln Leu Asp Asp Thr Ser Pro Arg Ala Ser Gly Gln Glu Thr Ser
1               5                   10                  15
Gln Ser Glu Tyr Asp Ala Arg Pro Leu Leu Ser Lys Phe Ala Ile Pro
          20                      25                  30
Arg Pro Trp Ser Arg
          35
```

(2) INFORMATION FOR SEQ ID NO:37:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Ile Asp Ser Ser Lys Asn Arg Ile Ser Gly Thr Gly Tyr Leu Ser Phe
1               5                   10                  15
Pro His Ile Arg His Ala Asn Arg Arg His Met Ala Asp Asp Ser Asn
          20                      25                  30
Leu Ala Pro Gly Pro Ser
          35
```

(2) INFORMATION FOR SEQ ID NO:38:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Trp Ser Ile Gly Thr His Thr Gly Pro Glu Gly Lys Phe Arg Ile Pro
1   5   10   15

Cys Asp Arg Ser Gly Cys Gly Gly Thr Thr Leu Thr His Gly Gly Leu
            20   25   30

Asn Ser Ser Pro Thr Gly Gln His Glu Arg Pro
            35   40

(2) INFORMATION FOR SEQ ID NO:39:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asp Pro Cys Glu Asp Gly Tyr Trp Leu Ser Ser Val Gly Arg Ala Gly
1   5   10   15

Ala Ser Ile Arg Gly Cys Gly Ala Ile Arg Arg Ser Ser Arg Thr Leu
            20   25   30

Thr Ala Glu Tyr Ser Thr Arg Ala Ser Asn His
            35   40

(2) INFORMATION FOR SEQ ID NO:40:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Gly Ser Lys Arg Ser Cys Trp Gly Thr Thr Ile Ser Asn Tyr Phe Arg
1   5   10   15

Pro Val Pro Glu Gly Cys Gly Ser Ala Ser Ser Ile Asn Pro Asn Thr
            20   25   30

Asn Thr Gly Arg Leu Pro Ser Leu His Arg Gln
            35   40

(2) INFORMATION FOR SEQ ID NO:41:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Ser Ser Ala Ser Ser Gly Cys Leu Gly Arg Ala Glu His Leu Asp Leu
1               5                   10                  15

Asp Ser Val Trp Gly Cys Gly Ser Gln Ala Asp Met Ser Arg Arg Tyr
            20                  25                  30

Ser Pro Trp Tyr Gly Arg Pro Arg Thr Gly Val
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:42:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Asn Val Met Trp Ser Ser Ser Lys Ala Gly Ile Arg Asp Cys Ser Gln
1               5               10                  15

Val Pro Pro Gly Gly Cys Gly Pro Val Asn Arg His Arg Ala Ser Pro
            20                  25                  30

Pro Leu Thr Pro Phe Arg His Gly Ser Ile Arg
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:43:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Pro Leu Thr Ser Gly Ser Ser Ser Glu Tyr Arg Asn Arg Asp Asp Cys
1               5                   10                  15

Pro Val Tyr Lys Tyr Ala Thr Asn Cys Pro Arg Leu Asn Phe Ser Pro
            20                  25                  30

Ser Arg Tyr Ser Pro Phe
        35
```

(2) INFORMATION FOR SEQ ID NO:44:

    (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

          Gly Asp Ala Tyr Gly Gly Ile Phe Ser Arg Pro Arg Gln Gly Leu Ala
          1   5   10   15

          Asp Ser Tyr Ile His Ala Ser Tyr Thr Gly Lys His Phe Phe Arg Gly
                      20   25   30

          Pro Arg Pro Pro Thr Arg
                  35

   (2) INFORMATION FOR SEQ ID NO:45:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

          Ser Thr Cys Ile Gly Ala Glu Gly Glu Trp Lys Ser Phe His Asn Phe
          1   5   10   15

          Leu Gln Cys Arg Asp Ala Thr Ser Thr Ser Ser Ser Thr Leu Asp Pro
                      20   25   30

          Thr Ala Leu Arg Phe Gly
                  35

   (2) INFORMATION FOR SEQ ID NO:46:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

          Tyr Ser Ala Thr Leu Trp Asp Gln Phe Gly Ser Arg Gln Val Glu Leu
          1   5   10   15

          Trp Ser Asn Arg His Ala Ser Ser Ala Leu Pro Phe Ala Ser Arg Ala
                      20   25   30

          Ser Val Leu Gly Ser Arg

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(A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

          Gly Asp Ala Tyr Gly Gly Ile Phe Ser Arg Pro Arg Gln Gly Leu Ala
          1   5   10   15

          Asp Ser Tyr Ile His Ala Ser Tyr Thr Gly Lys His Phe Phe Arg Gly
                      20   25   30

          Pro Arg Pro Pro Thr Arg
                  35

   (2) INFORMATION FOR SEQ ID NO:45:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

          Ser Thr Cys Ile Gly Ala Glu Gly Glu Trp Lys Ser Phe His Asn Phe
          1   5   10   15

          Leu Gln Cys Arg Asp Ala Thr Ser Thr Ser Ser Ser Thr Leu Asp Pro
                      20   25   30

          Thr Ala Leu Arg Phe Gly
                  35

   (2) INFORMATION FOR SEQ ID NO:46:

          (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 38 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: peptide

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

          Tyr Ser Ala Thr Leu Trp Asp Gln Phe Gly Ser Arg Gln Val Glu Leu
          1   5   10   15

          Trp Ser Asn Arg His Ala Ser Ser Ala Leu Pro Phe Ala Ser Arg Ala
                      20   25   30

          Ser Val Leu Gly Ser Arg

35

(2) INFORMATION FOR SEQ ID NO:47:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ile Leu Gly Trp Pro Phe Leu Thr Gly Leu Gly Asp Ser Thr Val His
1   5   10   15

Pro Arg Gly Arg Lys Gly Thr Asp Pro Ser
          20   25

(2) INFORMATION FOR SEQ ID NO:48:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser Ile Pro Ser Phe Ser Met Trp Leu Asn Gln Leu Gly Ser Ala Ala
1   5   10   15

Leu Pro Ser Lys Gly Asn Ser Gln Asp Arg Ser Asp
          20   25

(2) INFORMATION FOR SEQ ID NO:49:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ser Arg Asp Asp Ile Phe Thr Gly Gly Pro Leu Val Leu Phe Arg Gly
1   5   10   15

Ser Lys Thr Ser Asn His Asp Val His Ser Met Arg
          20   25

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 28 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Ala Glu Leu Val Asn Trp Tyr Glu Trp Phe His Val Thr Ala Glu
1   5   10   15

Ala Glu Thr Pro Val Ile Asn Ser His Asn Met Thr
            20   25

(2) INFORMATION FOR SEQ ID NO:51:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Ala Pro Val Trp Arg Gly Asn Pro Arg Trp Arg Gly Pro Gly Gly
1   5   10   15

Phe Lys Trp Pro Gly Cys Gly Asn Gly Pro Met Cys Asn Thr Phe Thr
            20   25   30

Pro Ala Arg Gly Gly Ser Arg Asn Asn Gly Pro
            35   40

(2) INFORMATION FOR SEQ ID NO:52:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gly Ser Ala Ser Ser Cys Phe Pro Asn Phe Thr Ala Arg Gly Val Thr
1   5   10   15

Val Gly Phe Phe Gly Cys Gly Ser Pro Ala His Pro Ala Ala Pro Arg
            20   25   30

Val Leu Asn Pro Ala Thr Asp Phe Pro Ala Pro
            35   40

(2) INFORMATION FOR SEQ ID NO:53:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Val Phe Arg Arg Thr Ala Arg Ser Ser Arg Pro Ile Gly Ala Thr Val
1   5   10   15

Phe Pro Trp Tyr Gly Cys Gly Asn Ser Asn Asp Glu Thr Leu Pro His
        20   25   30

His Asp Ser Pro Pro Ser Phe Phe Leu Gly Ala
       35   40

(2) INFORMATION FOR SEQ ID NO:54:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Asn Thr Cys Trp Thr Asp Leu Phe Trp His Gly Leu Pro Gly Gly Asp
1   5   10   15

Leu Pro Arg Asp Gly Cys Gly Leu Pro Ser Glu Leu Thr Thr His Pro
        20   25   30

Ser Arg Glu Arg Arg Asp Ala Ser Glu Asn
       35   40

(2) INFORMATION FOR SEQ ID NO:55:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Ile Asp Trp Asn Trp Leu Glu Arg Gly Gln His Asn Arg Gly Tyr Leu
1   5   10   15

His Ser Phe Pro Asp Ala Lys Ser Gln Pro Thr Arg Gly Pro Arg Val

20   25   30

          Ala Pro Asn Gly Asn Asp
                  35

(2) INFORMATION FOR SEQ ID NO:56:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 38 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

      Gly Arg Gly Ser Asp Met Arg Glu His Trp Pro Trp Ser Met Pro Leu
      1   5   10   15

      Ile Leu Asp Gln His Ala Asn Asp Pro Ser Pro Arg Ala Gln Ser His
                  20   25   30

      Tyr Tyr Ser His Pro Phe
              35

(2) INFORMATION FOR SEQ ID NO:57:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 43 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

      Val Ser Thr Gly Trp Ser Gly Thr Pro Arg Trp Cys Ala Pro Gly Gly
      1   5   10   15

      Lys Gln Gly Ser Gly Cys Gly Asn Gly Pro Arg Trp Thr Thr Leu Thr
                  20   25   30

      Pro Asp Leu Gly Gly Thr Arg Lys Tyr Gly Pro
              35   40

(2) INFORMATION FOR SEQ ID NO:58:

       (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 43 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: peptide

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
        Gly Ala Pro Leu Trp Cys Glu Lys Leu Ser Gly Thr Gly Ser Gly Gly
        1               5               10              15

        Phe Lys Trp Pro Gly Cys Gly Ser Gly Pro Thr Tyr Asn Thr Phe Thr
                        20              25              30

        Pro Ala Arg Val Gly Ser Asp Asn Lys Trp Pro
                35              40
```

(2) INFORMATION FOR SEQ ID NO:59:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
        Gly Pro Pro Val Trp Ser Ala Lys Ser Arg Trp Thr Gly Thr Gly Val
        1               5               10              15

        Leu Asn Trp Pro Gly Cys Gly Lys Val Pro Ser Cys Ser Thr Tyr Thr
                        20              25              30

        Pro Ser Arg Asp Arg Ser Arg Lys Ser Asp Pro
                35              40
```

(2) INFORMATION FOR SEQ ID NO:60:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
        Gly Ser Ala Leu Leu Thr Ser Lys Gly Cys Val Arg Gly Pro Gly Gly
        1               5               10              15

        Leu Met Arg Pro Gly Cys Gly Asn Asp Arg Leu Gly Lys Ser Ser Thr
                        20              25              30

        Tyr Ala His Gly Gly Trp Ile Lys Thr Gly Pro
                35              40
```

(2) INFORMATION FOR SEQ ID NO:61:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```
Gly Ser Pro Val Trp Ser Gly Asp Asn Arg Trp Arg Gly Ser Ser Pro
1               5               10              15
```

```
Leu Lys Arg Pro Gly Cys Gly Asn Gly Ala Lys Cys Asn Thr Leu Lys
        20              25              30
```

```
Asp Asn Arg Lys Asp Ser Arg Lys Thr Lys His
        35              40
```

(2) INFORMATION FOR SEQ ID NO:62:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```
Gly Pro Leu Leu Pro Gly Glu Ala Ala Val His Gly Ala Arg Gly Leu
1               5               10              15
```

```
Met Arg Ser Gly Cys Gly Asn Gly Pro Thr Trp Asn Arg Leu Thr Ala
        20              25              30
```

```
Ala Cys Arg Asp Ser Arg Asn Lys Gly Pro
        35              40
```

(2) INFORMATION FOR SEQ ID NO:63:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```
Gly Ser Pro Val Trp Met Gly Ser Thr Arg Trp Thr Gly His Gly Trp
1               5               10              15
```

```
Phe Arg Ser Gln Gly Cys Gly Asn Val Pro Arg Thr Asn Ser Cys Ala
        20              25              30
```

```
Pro Ala Gly Lys Asp Ser Gln Asn Lys Gly Pro
        35              40
```

(2) INFORMATION FOR SEQ ID NO:64:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

    Gly Ala Pro Val Trp Arg Gly Asn Arg Trp Cys Ser Asp Asn Gly Glu
    1   5   10   15

    Leu Glu Arg Pro Gly Cys Gly Tyr Gly Pro Arg Phe Asn Ile Leu Pro
                    20   25   30

    Pro Gly Arg Gly Asn Ser Arg Lys Pro Ser Pro
            35   40

(2) INFORMATION FOR SEQ ID NO:65:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

    Gly Ser Ser Gly Trp Lys Val Lys His Arg Cys Gly Gly Pro Gly Thr
    1   5   10   15

    Leu Gln Arg Pro Gly Cys Gly Asn Leu Pro Leu Gly His Thr Phe Pro
                    20   25   .   30

    Pro Thr Arg Gly Gly Ser His Met Glu Gly Ala
            35   40

(2) INFORMATION FOR SEQ ID NO:66:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

    Gly Pro Arg Ser Trp Met Gly Gln Pro Arg Gly Ser Asp Ala Gly Ser
    1   5   10   15

    Cys Lys Trp Ala Gly Cys Gly Asp Ala Pro Met Trp Arg Ala Ser Thr
                    20   25   30

    Pro Gly His Gly Gly Pro Pro Asn Arg Gly Ser
            35   40

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 43 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

     Glu Ala Leu Val Cys Arg Gly Lys Pro Pro Trp Ser Gly Pro Ala Gly
     1   5   10   15

     Leu Leu Trp Gln Gly Cys Gly Thr Gly Pro Val Ser Arg Thr Phe Thr
                 20   25   30

     Ser Ala Gln Gly Arg Ser Arg Asn Lys Thr Ser
             35   40

(2) INFORMATION FOR SEQ ID NO:68:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 43 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

     Gly Ala Pro Val Val Gly Asp Ile Leu Trp Cys Ser Gly Ala Arg Gly
     1   5   10   15

     Ala Lys Trp Pro Gly Cys Gly Lys Gly Pro Thr Asn Lys Thr Phe Ser
                 20   25   30

     His Ser Arg Gly Gly Thr Gln Lys Ser Gly Leu
             35   40

(2) INFORMATION FOR SEQ ID NO:69:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 43 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

     Gly Ala Pro Val Ser Arg Cys Lys Pro Ala Cys Gly Gly Phe Trp Gly
     1   5   10   15

     Val Asn Trp Pro Gly Cys Gly Asn Ala Ser Met Cys Lys Thr Phe Thr
                 20   25   30

```
         Asn Gly His Gly Val Ser Ser Asp Asn Gly His
              35                  40
```

(2) INFORMATION FOR SEQ ID NO:70:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

```
Gly Ala His Gly Tyr Lys Asn Gly Ser Thr Cys Thr Gly Leu Gly Gly
1               5                  10                  15

Trp Arg Cys Arg Gly Cys Gly Lys Gly Ala Met Cys Asn Asn Pro Ser
            20                  25                  30

Pro Ala Gly Gly Ala Tyr His Asn Gln Gly Pro
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:71:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

```
Gly Pro Gln Gly Ser Glu His Gln Cys Cys Ser Gly His Trp Gly Leu
1               5                  10                  15

Lys Phe Pro Gly Cys Gly Asn Gly Pro Ile Cys Asn Asn Phe Thr Ala
            20                  25                  30

Leu Arg Gly Ala Ser Arg Lys Asn Gly Pro
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:72:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```
Gly Glu Pro Val Trp Cys Arg His Ser Gly Gly Arg Val Gln Gly Gly
```

```
         1               5                    10                   15
     Leu Asp Trp Leu Gly Cys Gly Asp Gly Pro Leu Arg Tyr Thr Val Thr
                 20                  25                  30

     Pro Ala Arg Gly Gly Pro Ser Lys His Gly Pro
             35                  40
```

(2) INFORMATION FOR SEQ ID NO:73:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

```
     Gly Leu Ser Leu Val Arg Gly Asp Ser Trp Gly Ser Gly Ala Gly Gly
     1               5                    10                   15

     Trp Lys Arg His Gly Cys Gly His Gly Pro Met Tyr Asn Pro Gln Thr
                 20                  25                  30

     Pro Ala Arg Gly Gly Ser Cys Thr Arg Asn Thr
             35                  40
```

(2) INFORMATION FOR SEQ ID NO:74:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

```
     Val Ser Arg Ala Trp Ser Gly Lys Pro Arg Leu Met Gly Ser His Gly
     1               5                    10                   15

     Leu Asn Cys Pro Gly Cys Gly Lys Gly His Ser Gly Ile Met Phe Ile
                 20                  25                  30

     Pro Asp Pro Ala Gly Ser Ala Asn Thr Pro Pro
             35                  40
```

(2) INFORMATION FOR SEQ ID NO:75:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TCPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Cys Ala Pro Met Trp Ser Gly Lys Pro Pro Trp Cys Val Gly Gly Gly
1   5   10   15

Val Lys Phe Arg Gly Cys Gly Asn Arg Pro Asp Cys Asn Ile Ile Thr
            20   25   30

Pro Arg Leu Val Glu Ser Arg Asp Lys Ala Leu
        35   40

(2) INFORMATION FOR SEQ ID NO:76:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Ala Asp Pro Val Cys Ser Arg Lys Pro Asp Gly Gly Gly Leu Arg Gly
1   5   10   15

Leu Arg Trp Pro Gly Cys Gly Lys Gly Pro Ile Leu Tyr Asn Val Thr
            20   25   30

Ala Ala Arg Gly Gly Ser Arg Asn Asn Gly Pro
        35   40

(2) INFORMATION FOR SEQ ID NO:77:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Gly Thr Arg Val Pro Pro Gly Phe Ala Leu Arg Gly Gly Arg Asp Gly
1   5   10   15

Leu Ser Trp Ala Gly Cys Gly Lys Ala Pro Ile Ser Lys Thr Tyr Thr
            20   25   30

Ser Ala Arg Gly Arg Ser Arg Lys Lys Gly Ser
        35   40

(2) INFORMATION FOR SEQ ID NO:78:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Arg Ser Ala Val Ser Glu Gly Lys Pro Arg Glu Ile Val Pro Gly Gly
1   5   10   15

Cys Met Trp Pro Gly Cys Gly Asn Gly Arg Lys Ser Asn Thr Leu Thr
            20   25   30

His Gly Pro Glu Gln Phe Gln Glu Ile Glu Pro
    35   40

(2) INFORMATION FOR SEQ ID NO:79:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Ser Ser Gly Val Gly Asn Gly Lys Pro Arg Ser Trp Ala Pro Asp Ala
1   5   10   15

Leu Asn Gly Gly Cys Gly Asn Ile Gln Phe Ala Asn Thr Ile Thr Pro
            20   25   30

Asp Arg Gly Gly Ser Cys Asn Gln Thr Leu
    35   40

(2) INFORMATION FOR SEQ ID NO:80:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Gly Ser Ser Val Cys Gly Gly Gln Pro Ser Gly Arg Gly Phe Gly Gly
1   5   10   15

Leu Pro Gly Pro Gly Cys Gly Asn Gly Pro Thr Ser Asn Thr Leu Thr
            20   25   30

Ser Ala Arg Gly Gly Phe Pro Asn Lys Gly Leu
    35   40

(2) INFORMATION FOR SEQ ID NO:81:

    (i) SEQUENCE CHARACTERISTICS:

-110-

        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

    Gly Ala Pro Leu Trp Gln Gly Asp Pro Ala Asp Glu Val Leu Gly Gly
    1   5   10   15

    Ser Met Ile Pro Gly Cys Gly Ile Gly Ala Leu Ser Gln Thr Phe Thr
                20   25   30

    Pro Thr Pro Gly Gly Ser Arg Lys Asn Val Thr
            35   40

(2) INFORMATION FOR SEQ ID NO:82:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

    Ala Gly Arg Glu Leu Arg Gln Asp Glu Gly Glu Gly Gly Ala Gly Ala
    1   5   10   15

    Asp Val Ala Arg Leu Arg Glu Gly Pro Ile Cys Ser Thr Phe Thr Pro
                20   25   30

    Ala Arg Gly Gly Ser Cys Pro Ser Gly Leu
            35   40

(2) INFORMATION FOR SEQ ID NO:83:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

    Gln Ala Arg Val Ser Met Ala Ile Ser Cys Arg Ser Gly Pro Ser Asp
    1   5   10   15

    Leu Met His Gln Gly Cys Gly Tyr Gly Pro Arg Cys Asn Pro Asp Thr
                20   25   30

    Thr Asp Ser Gly Gly Ser His Thr Asn Thr Pro

(2) INFORMATION FOR SEQ ID NO:84:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Gly Asp Pro Glu Cys Arg Gly Lys Pro Arg Gly Arg Trp Thr Gly Ser
1   5   10   15

Leu Ala Cys Thr Gly Cys Gly Asn Gly Pro Asn Ser Lys Ile Cys Thr
                20   25   30

Arg Ala Arg Gly Val Ser Arg Asn Lys Gly Pro
            35   40

(2) INFORMATION FOR SEQ ID NO:85:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Ser Thr Pro Gly Cys Ser Gly Tyr Ser Gly Ser Gly Asp Pro Arg Cys
1   5   10   15

Leu Thr Cys Thr Ala Cys Gly Asn Gly His Thr Arg Lys Thr Leu Thr
                20   25   30

Pro Ala His Gly Arg Ser Thr His Lys Glu Pro
            35   40

(2) INFORMATION FOR SEQ ID NO:86:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 43 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Gly Gln Pro Glu Cys Arg Ile Thr Ser Gly Cys Cys Gly Thr Asp Gly
1   5   10   15

```
        Asn Lys Trp Leu Gly Cys Gly Lys Val Asp Met Cys Asn Thr Leu Asn
                    20                  25                  30

        Pro Ala Val Gly Cys His Gly Thr Asn Gly Ser
                35                      40
```

(2) INFORMATION FOR SEQ ID NO:87:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 56 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

CTGTGCCTCG AGBNNBNNBN NBNNBNNBNN BNNBNNBNNB NNBNNBNNBN CCGCGG   56

(2) INFORMATION FOR SEQ ID NO:88:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 56 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CTGTGCTCTA GAVNNVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN CCGCGG   56

(2) INFORMATION FOR SEQ ID NO:89:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 49 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

TCGAGBNNBN NBNNBNNBNN BNNBNNBNNB NNBNNBNNBN NBNCCGCGG   49

(2) INFORMATION FOR SEQ ID NO:90:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 49 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CTAGTVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNCCGCGG   49

(2) INFORMATION FOR SEQ ID NO:91:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 5
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser or Arg"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 18
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Pro, Thr, or Ala"

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 20
        (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Val, Ala, Asp, Glu, or Gly"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Ser His Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1   5   10   15

Xaa Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            20   25   30

Ser Arg Pro Ser Arg Thr
            35

(2) INFORMATION FOR SEQ ID NO:92:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 81 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GTGTGTCTCG AGNNNBNNBN NBNNBNNBNN BNNBNNBNNB NNBNNBNNBN NBNNBNNBNN   60

BNNBNNBNNB NNBNACGCCA N   81

(2) INFORMATION FOR SEQ ID NO:93:

-114-

(i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 66 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GTTGTGTCTA GAVNNVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNNVNNVNT   60

GGCGTN   66

(2) INFORMATION FOR SEQ ID NO:94:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 74 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TCGAGNNNBN NBNNBNNBNN BNNBNNBNNB NNBNNBNNBN NBNNBNNBNN BNNBNNBNNB   60

NNBNNBNACG CCAN   74

(2) INFORMATION FOR SEQ ID NO:95:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 59 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CTAGAVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNNVNNVNN VNTGGCGTN   59

(2) INFORMATION FOR SEQ ID NO:96:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 44 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (ix) FEATURE:
            (A) NAME/KEY: Modified-site
            (B) LOCATION: 4

(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser or Arg"

  (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 25
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Tyr, His, Asn or Asp"

  (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION: 27
              (D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ile, Met, Thr, Asn, Lys, Ser, or Arg"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

  His Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
  1   5   10   15

  Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa
              20   25   30

  Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Arg
          35   40

(2) INFORMATION FOR SEQ ID NO:97:

    (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 82 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GTGTGTCTCG AGNNNBNNBN NBNNBNNBNN BNNBNNBNNB NNBNNBNNBN NBNNBNNBNN   60

BNNBNNBNNB NNBGGTTGTG GT   82

(2) INFORMATION FOR SEQ ID NO:98:

    (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 81 base pairs
              (B) TYPE: nucleic acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GTTGTGTCTA GAVNNVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNNVNNVNN   60

VNNVNNVNNV NNACCACAAC C   81

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 75 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

TCGAGNNNBN NBNNBNNBNN BNNBNNBNNB NNBNNBNNBN NBNNBNNBNN BNNBNNBNNB   60

NNBNNBGGTT GTGGT.   75

(2) INFORMATION FOR SEQ ID NO:100:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 74 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CTAGAVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNNVNNVNN VNNVNNVNNV   60

NNVNNACCAC AACC   74

(2) INFORMATION FOR SEQ ID NO:101:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 49 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (ix) FEATURE:
            (A) NAME/KEY: Modified-site
            (B) LOCATION: 4
            (D) OTHER INFORMATION: /label= Xaa
    /note= "Xaa = Ser or Arg"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

    His Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    1   5   10   15

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Cys Gly Xaa Xaa Xaa Xaa Xaa
            20   25   30

    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser
        35   40   45

    Arg

                                -117-

(2) INFORMATION FOR SEQ ID NO:102:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 69 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GGSGCSCCSG TSTGSAGSGG SAASCCSCGS TGSAGSGGSC CSGGSGGSTT SAASTGSCCS   60

GGCTGCGGG   69

(2) INFORMATION FOR SEQ ID NO:103:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 69 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear .

   (ii) MOLECULE TYPE: DNA

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

SGGSCCSTTS TTSCGSGASC CSCCSCGSGC SGGSGTSAAS GTSTTSCASA TSGGSCCSTT   60

CCCGCAGCC   69